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JOURNAL OF DAIRY SCIENCE

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

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Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510–522 North Hickory Street, Champaign, Illinois.

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SUMMARY

TERRAMYCIN IN MILK PRODUCTION
AVERAGE OF 17 TRIALS (186 DAYS)

	Control	Treated
Number of cows	1193	1177
Av. days on experiment [1]	186	186
Av. daily milk prodn./cow, lb.	30.98	31.85
Increase in daily production, lb.	—	0.87
Increase, %	—	3
Extra milk per cow, lb.	—	161.8
Value of extra milk, $[2]	—	8.09
Cost of Terramycin per cow, $[3]	—	1.26
Extra return per cow, $	—	6.83

[1]Range 119-280 days [2]Milk valued at $5 per cwt.
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PEOPLE AND EVENTS

Dr. J. A. Nelson Retires at Montana State

J. A. NELSON retired from active duty July 1, 1961, and was granted the title of Professor Emeritus after 39 years of service at Montana State College.

Dr. Nelson came to Montana State College in 1922 as instructor in dairy manufacturing. In 1929, he was given the rank of professor and head of the newly created Department of Dairy Industry. The Department was enlarged in 1938 to include dairy production and the research work was greatly expanded. In 1948, he was appointed the first graduate dean of the newly created Graduate Division. He held both executive positions until 1955 when, on his request and due to age limit, he was relieved from the Graduate Division, but continued as department head. In 1956, on his request, he was relieved as department head and has continued since that time as Professor of Teaching and Research.

J. A. Nelson

Dr. Nelson received the B.S. degree from Iowa State University in 1922, the M.S. in 1923, and the Ph.D. degree from the same institution in 1932. He was very active in A.D.S.A., serving as chairman of the Western Division, active on many committees. Vice-President in 1945 and President in 1946, and received the first Award of Honor of that organization in 1959. He was also active in other scientific organizations.

He worked his way through college, graduating with honors and was elected to Phi Kappa Phi, Gamma Sigma Delta, and Alpha Zeta. As a graduate student, he was elected to Sigma Xi. Later, he was honored by being listed in American Men of Science, Leaders in American Science, Who Is Who in American Education, Who Is Who in the West, Who Knows and What, and Who Is Who in America. He is a member of the Dairy Shrine Club and is active in the Methodist Church and in civic affairs.

During 1960 Dairy Industry Week, which Dr. Nelson inaugurated in 1933 at Montana State College, he was honored by staff members, students, and men from the industry throughout the State. (J. Dairy Sci., 44:3. 1961.)

Dr. Nelson is co-author of a text on Judging Dairy Products, has done considerable research work in the fields of flavor defects and improvement of flavor in dairy products. He is author or co-author of some 50 bulletins, circulars, and articles on dairy products, dairy production, and kindred subjects. He has been granted office space in the department, to be used at his pleasure. He intends to spend his leisure time writing and in continued service to the dairy industry. He resides at 1110 5th Avenue, Bozeman, Montana.

Professor R. E. Horwood retires

R. E. HORWOOD, a member of the staff of the College of Agriculture of Michigan State University since June, 1927, has retired.

Professor and Mrs. Horwood will make their home at Lake City, where he lived from 1957 through June of 1960. During this time he was superintendent of the Lake City Experiment Station and supervisor of Extension demonstrations in northern Michigan.

R. E. Horwood

Professor Horwood's first position with Michigan State in June, 1927, was as Extension Specialist in dairy in the upper peninsula. He returned to the MSU campus in 1938 to do teaching and research in dairy. For a year during 1946-1947 he was on a special assignment with the U.S. Army, to help organize an Extension Service in Japan.

From 1949 to 1956 he was district Extension supervisor in the upper peninsula, with headquarters at Marquette, and superintendent of the upper peninsula Experiment Station at Chatham. Two years, 1951-1953, of this time he was called to a special assignment to direct Michigan State University mission at the University of Ryukyus in Okinawa.

Shortly after returning to the MSU campus at East Lansing in July, 1960, Professor Horwood suffered a heart attack and retired by action of the State Board of Trustees due to health reasons after 34 years' service.

Fred R. Evans Retires

F. R. EVANS, bacteriologist, Dairy Products Laboratory, Eastern Utilization Reserach and Development Division, retired May 12 after 43 years of continuous service in the United States Department of Agriculture. Following graduation from Cornell in 1918, he worked under Dr. L. A. Rogers on the bacteriology of condensed and evaporated milks. From 1922 to 1935, he was bacteriologist at the Bureau

of Dairy Industry Experimental Creamery at Grove City, Pennsylvania. During this period he worked with associates on the bacteriology of Swiss Cheese starters, the effects of milk quality on cheese quality, and resazurin as an indicator of milk quality. The latter was the first work of its kind in this country and was the basis for the present resazurin test.

When the pilot plant was moved to Beltsville, Maryland, in 1935, Mr. Evans returned to the Washington laboratory, where he worked for a short time with Dr. Rogers on the use of trisodium phosphate for cleaning dairy equipment. Later he worked with Drs. H. R. Curran and R. W. Bell on time-temperature relationships for high temperature-short time sterilization of evaporated milk.

During the past 25 years, Mr. Evans has been the able junior partner of Dr. Curran in basic research on bacterial spores. The work of this pair on the effects of physical and chemical agents on spores has been varied and voluminous. It has received world-wide recognition for its contribution to fundamental knowledge of spore germination and spore destruction.

Dr. George P. Gundlach Retires

G. P. GUNDLACH, a native of Tiffin, Ohio, was born October 20, 1891, the son of Anthony W. and Louisa Matilda Gundlach. His maternal grandfather, Francis Reif, who settled in Tiffin in 1835, was a pioneer there.

G. P. Gundlach

Following elementary and high school in Tiffin, Dr. Gundlach pursued special studies at Heidelberg College at Tiffin and later enrolled in Ohio State University, pursuing special courses in chemistry, bacteriology, and mathematics/sciences. During his OSU career, Dr. Gundlach affiliated with the Alpha Tau Omega fraternity and through the years has been active in alumni interests.

Upon leaving Ohio State, Dr. Gundlach became associated with the A.C. Spark Plug Division of The General Motors Corporation in Flint, Michigan.

Applying fruits of his technical and scientific professional education, Dr. Gundlach in 1919 became associated with The Frederick C. Mathews Company, Detroit, and during his career there as a dairy consultant was engaged in research, writing, lecturing, and counsellor work, especially in pasteurization of milk. Here he was associated with Dr. H. A. Harding, who had some years before been head of the Department of Dairy Husbandry at the University of Illinois.

Later, going to Dayton, Ohio, Dr. Gundlach pursued his career, prior to coming to Cincinnati in 1931, founding the dairy consultant firm bearing his name, G. P. Gundlach & Company. From a modest beginning the Gundlach firm has attained world-wide recognition.

Gifted with ability to transmit knowledge to others, Dr. Gundlach has had facile faculties of both speech and pen to make his talents available to others.

In collaboration with Thurman B. Rice, M.D., of Indiana University, Bloomington, Dr. Gundlach provided the advisory and consultant material for The Nutritional Needs of Adults, representing a valuable contribution in the field of public health and nutrition.

Dr. Gundlach's reputation was also recognized in 1959, when the Governor of Ohio designated him to represent that state in the important International Conference of the World Dairy Congress convened in London. In 1953, Dr. Gundlach was an invited speaker participant in the World Dairy Congress held at The Hague.

Recipient of the honorary degree of Doctor of Humane Letters from Heidelberg College, Tiffin, Ohio, in 1960, in recognition of his wide attainments in contribution to the dairy industry and its related developments to modern health, nutrition, and living, Dr. Gundlach, due to numerous pioneering pursuits, has also attained an industry salute as Mr. Dairyman.

He is married to the former Florence Baker. They have one son, G. B. Gundlach, who succeeded his father as president of the Gundlach firm.

UNIVERSITY NEWS

California

Dr. L. M. Smith to Study in New Zealand

A Fulbright award has been granted L. M. SMITH, Associate Professor of Food Science and Technology, University of California, Davis, who will conduct research at the Fats Research Laboratory of the Department of Scientific and Industrial Research, Wellington, New Zealand. His study will be concerned with the chemistry of animal fats, with particular reference to the nature of the phospholipids found in edible fats present in foods exported from New Zealand. During his year's stay in New Zealand, Dr. Smith will attend

L. M. Smith

a number of scientific meetings and will visit the New Zealand Dairy Research Institute. Dr. and Mrs. Smith and their two sons expect to sail from San Francisco on the maiden voyage of the Pacific and Orient Lines' Canberra, late in July.

Illinois

Program for Milk Concentrates Conference

The Department of Food Technology at the University of Illinois will be host to the Fifth Milk Concentrates Conference October 18 to 19, 1961. The program is completed. The various subjects selected were suggested by industry and by federal and state experiment station investigators.

On the first day of the conference, Dr. M. J. Pallansch and H. J. Sinnamon will present a paper on the production and properties of dry whole milk foams; Dr. Stuart Patton will discuss off-flavors that develop in dry whole milk powder; and Dr. Mark Keeney will speak on this same subject for nonfat dry milk powder. Some facts about the stability of concentrated sterile milk will be presented by Dr. M. E. Ellertson and S. J. Pearce. Protein-salt and protein-protein reactions in concentrated milk products will be discussed by Dr. C. A. Zittle, and Dr. J. R. Brunner will continue this subject for protein-fat reactions.

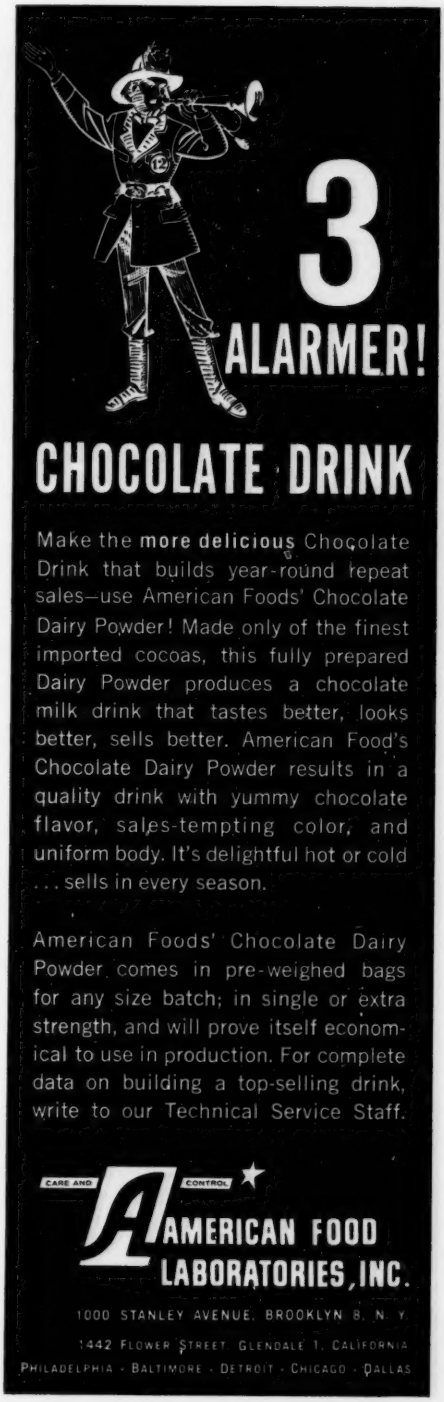
For the second day of the conference, Dr. Henry Klostergaard will speak on new concepts about the hydration of milk proteins. The effect of heat on the components of casein and on hydrogen bonding will be discussed by Dr. W. G. Godbey. Methods for evaluating the heat treatment of low-heat powder will be discussed by Dr. S. T. Coulter. Developments on the stability of frozen whole and concentrated milk will be presented by Dr. W. C. Winder. Dr. E. V. Jensen of the University of Chicago will discuss the effects of heat on the sulfur systems in milk.

It is planned to schedule three papers for part of each half-day. During the remainder of this period, questions will be directed toward a panel of scientists, consisting of the speakers and several other outstanding authorities who have established competence in the subjects presented by the speakers.

Iowa

Dedication of National Animal Disease Laboratory

A new era of hope that serious losses from animal diseases in the United States can be eliminated began with the dedication of the U. S. Department of Agriculture's new $16\frac{1}{2}$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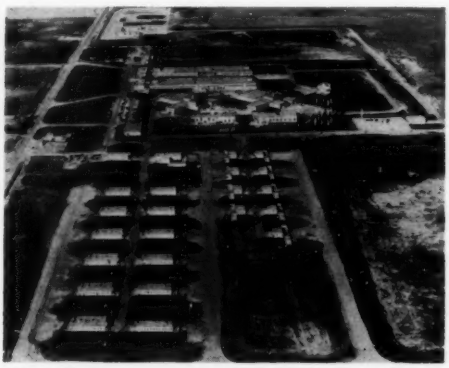
million National Animal Disease Laboratory at Ames, Iowa, June 27, 1961.

The new facility, the best of its kind anywhere, will enable scientists of the Department's Agricultural Research Service to serve with fresh expectations in their historic role as guardians of the health of the nation's livestock.

The new Ames research center represents USDA's second major step in its effort to modernize animal disease research in the United States. The first was the opening in 1956 of the $10 million laboratory at Plum Island, L. I., N. Y., for research on foreign diseases that threaten U. S. livestock. Research on animal parasites will continue at USDA's Agricultural Research Center, Beltsville, Md., Auburn, Ala., and other field



National Animal Disease Laboratory at Ames, Iowa

locations where it has been conducted for many years. Animal disease research facilities in Washington, D.C., Beltsville, and Auburn, however, are being replaced by the new national laboratory.

The Ames laboratory will serve a great need in a changed world. In spite of advances in veterinary science and gains in knowledge of animal diseases since USDA, in 1883, first undertook to do battle against them, the fight is becoming an increasingly difficult one.

The very efficiency that has been developed through research for the U. S. livestock industry creates its own difficulties. For example, the incidence of bovine tuberculosis is low enough to take pride in, but still high enough to be dangerous. The remaining traces of the disease are now difficult to detect and wipe out. The same is true for many other animal and poultry diseases.

Fincham Named Assistant Manager of Iowa Cooparative

R. C. FINCHAM, extension dairyman at Iowa State University for 14 years, has accepted the position of assistant manager of Iowa Breeders Cooperative, effective July 1. He will be in charge of public relations and education, and his primary duties will involve working with about 25,000 cooperative members in developing more profitable livestock programs.

R. C. Fincham

Professor Fincham has been active on the Program Committee of the Mid-West Conference on Artifical Insemination. As a member of the American Dairy Science Association he has served on the Breeding Committee, the Breeds Relations Committee, and the Teaching Methods Committee.

For 12 years he has been superintendent of dairy cattle at the Iowa State Fair and has judged many area shows, which he will continue in his new position.

A native Iowan, Fincham received both B.S. and M.S. degrees from Iowa State University, and served as county extension director in Emmet County and as 4-H Club Agent in Wright and Hamilton counties before entering dairy husbandry extension work at Iowa State.

He and his family will continue to reside at Ames.

Ohio

Recent additions to the staff of the Department of Dairy Technology, The Ohio State University, include the following: as Assistant Professor in the University and Research Associate, Ohio State Research Foundation, DR. E. M. MIKOLAJCIK and DR. C. V. MORR; and as Research Associate, Ohio Agricultural Experiment Station, M. E. OLDHAM.

Dr. Mikolajcik received his advanced degrees from Ohio State, and returns to the University after six years on the faculty of The University of Puerto Rico. He will carry out research and teaching responsibilities in the area of dairy bacteriology.

Dr. Morr received his graduate and undergraduate training at Ohio State. For the past two years he has been associated with the Research Division of The Carnation Company, Van Nuys, California. His research specialty at Ohio State will be the field of milk proteins.

Mr. Oldham comes to the Department as a Doctoral candidate and will conduct cheese research. He received the B. S. and M.S.

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degrees from Utah State University, following which he served on the faculty of the University of Wyoming.

The Ohio State Fair will be held in Columbus from August 25 to September 1. Staff members of the Department of Dairy Technology, Ohio State University, participate actively each year in those aspects of the fair dealing with dairy products. The Department cooperates with the Ohio Dairy Products Association in arranging for exhibits in the Dairy Products Building, which are designed to acquaint visitors with the diverse nature of the field of Dairy Technology.

DR. T. KRISTOFFERSEN serves as Superintendent of the Dairy Products contests, which include Swiss cheese, vanilla, chocolate, and strawberry ice cream, and Cottage cheese. DR. M. E. GREGORY, Extension Specialist in Dairy Technology, is in charge of the F.F.A. quality milk contest, in which teams representing F.F.A. chapters throughout the state compete in demonstrating their ability to produce milk of high quality. The contest involves the submission of milk samples for bacterial and compositional analysis and a written examination testing knowledge of dairy farm management. The winning team is awarded a trip to Chicago, to visit dairy industrial facilities there.

Oregon

Second Symposium Planned at State University

Plans have been announced by Oregon State University for its second biennial Symposium on Foods, September 11 to 13. Theme for the symposium is Oxidative Deterioration of Food Lipids. Food scientists from across the nation and from several foreign countries will be participating, according to DR. H. W. SCHULTZ, head of the university's department of food and dairy technology.

DR. C. H. LEA of the University of Cambridge, England, will be opening speaker for the three-day meetings. Other speakers from outside the United States will include: K. U. INGOLD, National Research Council, Ottawa, Canada; G. HOFFMANN, Unilever Research Laboratory, Vlaardingen, The Netherlands; and NOBORU MATSUO, Seikei University, Tokyo, Japan.

Sixteen U.S. leaders will be speakers: W. O. LUNDBERG, J. R. CHIPAULT, The Hormel Institute, University of Minnesota; E. N. FRANKEL, Procter and Gamble Co., Cincinnati, Ohio; MARK KEENEY, University of Maryland; A. L. TAPPEL, H. S. OLCOTT, J. F. MEAD, University of California; STUART PATTON, Pennsylvania State University; BETTY M. WATTS, Florida State University; R. B. KOCH, Minneapolis-Honeywell Regulator Co., Hopkins, Minn., L. J. MACHLIN, Monsanto Chemical Co., St. Louis, Mo.; HANS KAUNITZ, Columbia University; C. G. KING, Nutrition Foundation Inc., N.Y.; F. A. KUMMEROW, University of Illinois; KLAUS SCHWARZ, National Institutes of Health, Bethesda, Md.; and B. N. STUCKEY, Eastman Chemical Products Inc., Kingsport, Tenn.

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STUDENT CHAPTER NEWS

J. E. JOHNSTON, Editor

A Section Devoted to News of Student Members

The Student Branch—A.D.S.A. Annual Meeting June 11-14, 1961

Launching the National Student Branch, like launching our first astronaut, required that many people devote their skills and services. The success of the meeting at Madison is indeed a gratifying tribute to all who have made this launch possible.

Two luncheons were held, which enabled over 50 student affiliates to meet with more than 20 outstanding men of the Association. Through close contact fostered by eating together, many young leaders were able to gain from the experiences of our senior members. This year, as last year, it was acclaimed a most worthwhile function.

In the general meetings many items were discussed and a number of motions passed. The major motions approved by the delegates that will affect all members are: (1) To cover the necessary operating expenses of the National Student Branch, each affiliated chapter will be charged $5 annually, payable to the Secretary-Treasurer on or before the date the annual reports are due (May 15, 1962). (2) The membership adopted an emblem. President Lineweaver had designed, obtained approval, and investigated the cost of using it in various ways as part of the wearing apparel. Due to cost considerations, this discussion was tabled until next year; however, it was suggested that the emblem be incorporated into a lapel pin or tie pin.

Undoubtedly, our most successful and enlighting function at the Annual Meeting was the symposium Selecting a Career in the Dairy Industry. Thanks to the wisdom and experience of each participant, we all learned of

new opportunities in Education and Research, Foreign Service, Production, and Manufacturing.

Congratulations are in order to the Georgia Chapter for winning the 1961 Outstanding Student Affiliate Chapter Award. There were many excellent annual publications displayed this year; however, it should be noted that the judges' score-card for evaluating the total activities of the club lists other important points which involve the work of the entire club and not just the reporters.

The new officers, selected to guide the National Student Branch during the coming year, are: Joseph Lineweaver, President, Virginia; Jacob Megehee, First Vice-President, Mississippi; Keith Jenkins, Second Vice-President, Ohio; Dennis Boesiger, Third Vice-President, Nebraska; and Camilla Cristman, Secretary-Treasurer, Michigan.

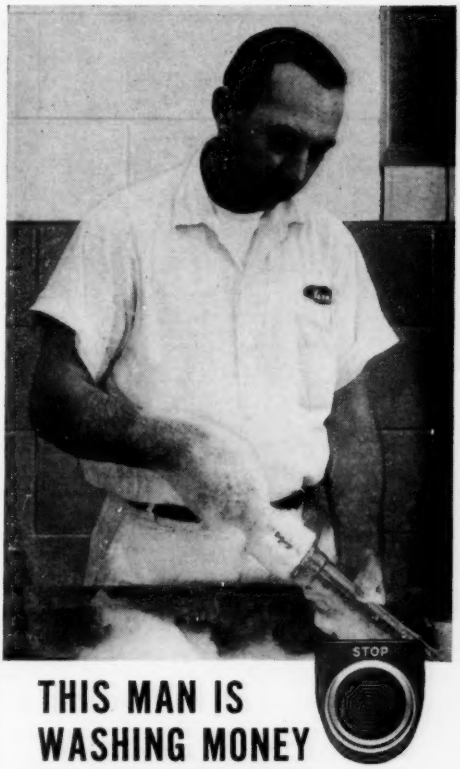
At the close of the meetings, the newly elected and the retiring officers closely examined this year's activities and laid plans for next year's program at Maryland.

CAMILLA CRISTMAN
Secretary-Treasurer

The Student Chapter Award

The Outstanding Chapter Contest had 16 entries this year, with a number of excellent exhibits in the form of scrapbooks and chapter annuals. The judges were: T. P. Head of the Progressive Farmer; John Speer of the International Association of Ice Cream Manufacturers; and Robert Morton of Ralston-Purina Co. The winner of the 1st-place plaque and $100 award was the Dairy Science Club of the University of Georgia. Second place was won by the Virginia Tech. Chapter, A.D.S.A., which came very close to repeating its 1960 victory. There was a third-place tie, with the Dairy Tech. Club of Ohio State University and the Penn State Chapter, A.D.S.A., so close that the judges could not separate them. Second- and third-place winners received a certificate of merit and an honorable mention certificate, respectively, and other entries received certificates of participation.

The rules of this contest specify that to qualify its entry each chapter must have its annual report in on time and must present an exhibit at the annual meeting. The exhibit may consist of a scrapbook, an annual, or both. The judging is based in part on the quality of the exhibit and in part on the evidence of outstanding club activity presented in the annual report and the exhibit. In evaluating evidence of chapter activity the size of the club and the number of departmental majors are taken into account, to insure that each chapter has an equal opportunity of winning. This award will be presented again at the 1962 convention. The official rules will be distributed to the chapters in the near future.



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Summary of 1960-61 Student Chapter Activities

JOSEPH A. LINEWEAVER

One meeting of the National Student Branch, at the 56th Annual Meeting of A.D.S.A., was set aside for a summary of the outstanding chapter activities during the past year. This program was conducted for the purpose of sharing ideas that will help all chapters become more active and effective in their annual programs.

Chapter annual reports were reviewed and the activities to be reported at the national meeting were assigned each chapter prior to the meeting, to avoid two chapters reporting on the same activity. This is the reason for the May 15 deadline for the annual reports. The annual reports are most satisfactory when set up in the suggested outline form rather than as an essay on the year's activities.

The annual programs of most of the chapters follow the same general pattern. The school year is usually started with an informal get-acquainted picnic for old members, new members, and faculty. The meetings during the school year are usually dotted with guest speakers, movies, and slides, and the year is concluded with an awards or honors banquet.

Each chapter is different and unique in balancing out the open spaces in this general outline. These additional activities are the ones all of us should work on for the coming years.

Following is a list of some of these activities for the past year reported in the annual reports.

A. Formal programs:
   1. Speakers from the dairy industry and allied fields.
   2. Films and slides
   3. Initiation of new members.

B. Educational programs:
   1. Erect displays for livestock shows.
   2. Homecoming displays and floats.
   3. Dairy or Agricultural Expositions, Dairy Shows, Farmers Week, etc.
   4. Local products judging contests and dairy cattle judging contests.
   5. Sponsor products and cattle judging teams to regional and national contests.

C. Fund-raising activities:
   1. Livestock sales.
   2. Sponsor rodeo.
   3. Department donate a percentage of the selling price of heifers to chapter.
   4. Serve luncheons and meals.
   5. Sell cull cows after processing.
   6. Slaughter and sell a steer.

7. Cheese sales at Thanksgiving, Christmas, and Easter.
8. Buy and sell heifer calves.
9. Refreshment stands at fairs.
10. Vending machines at school.
11. Raffles.

D. Socials:
1. Picnics.
2. Homecoming breakfasts or similar activity for alumni.
3. Dinner dances.
4. Awards or honors banquet.

E. Publications:
1. Chapter annuals.
2. Newsletters.
3. Scrapbooks and annual reports.
4. News articles in school, local, and area newspapers.
5. News articles in student section of the JOURNAL OF DAIRY SCIENCE.

F. Special projects:
1. Career day for prospective students.
2. Club trips.
3. Christmas party for needy children.
4. Aid in FFA and 4-H judging contests.
5. Ice cream–eating contest.
6. Milk-drinking contest.
7. Outstanding senior award.
8. Hall of Fame or Dairy Club Achievement Award for outstanding alumni or men in the dairy industry in the state.
9. Dairy club Princess contest.
10. Donations to charity.

This is a brief summary of the annual activities of many chapters throughout the nation. In evaluating your chapter's annual program, this report may aid in helping you create a more rounded and more effective program for the coming year.

Organize Potomac Dairy Analysts

A group of consultants and analysts from Maryland, Virginia, and the District of Columbia have organized the Potomac Dairy Analysts. The objectives of this organization are: 1. To be of mutual benefit to its members. 2. To broaden their knowledge in the scientific aspects of dairy product analyses. 3. To bring about greater uniformity in analytical procedures.

Membership is confined to those persons only who are actively engaged in the analyses of dairy products, teaching, and regulatory work involving dairy products.

The officers of this association are: President: J. H. LLOYD, JR., Sealtest Foods, Eastern Division; Vice-President: C. M. MECHAM, Maryland and Virginia Milk Producers Association; and Secretary-Treasurer: DR. C. W. ENGLAND, The C. W. England Laboratories.

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INDUSTRY NEWS

American Can Company Establishes New Division

A fully staffed sales organization that will devote all its efforts solely to the service of the dairy industry has been established by the new Milk Container Division of American Can Company.

All sales of containers and filling equipment will be the full responsibility of the new Division. Each Division man not only will sell, but will provide full plus services to the dairy and juice industries, ranging from assistance at the plant to point-of-sales merchandising of the product.

Each man in the sales department has had years of experience in practical packaging. Each is abreast of current merchandising trends and sales problems of the dairy and juice industries. And through the field sales force, all customers will have direct access to all the broad technical, manufacturing, and research facilities of American Can Company.

E. E. MOORE has been elected vice-president and general manager of the American-Wheaton Glass Corp., a majority-owned subsidiary of the American Can Company. A minority interest is held by the Wheaton Glass Company. American-Wheaton was organized in 1960 to produce glass containers for food.

E. M. Muldoon Elected Vice-President Beatrice Foods

E. M. MULDOON has been elected a vice-president of Beatrice Foods Co. He will continue in his present position as general manager of the Grocery Products Division of Beatrice Foods and general manager of La Choy Food Products, Archbold, Ohio.

E. M. Muldoon

Muldoon has served in virtually every capacity in the food industry for 35 years. He started delivering groceries in his hometown of Youngstown, Ohio, as a boy. Following graduation from the University of Michigan School of Business, he was with one of the nation's largest food chains for 21 years.

His experience includes operations on the retail, wholesale, and manufacturing levels in such capacities as store manager, office manager, divisional auditor, buyer, advertising manager, sales manager, merchandising manager, director of sales, vice-president, and general manager and executive vice-president.

ANIMAL PRODUCTION

Journal of The British Society of Animal Production

Contents of Volume 3, Part 3, October 1961

ALBA, J., CORTA, E.V. and ULLOA, G., Influence of natural service on length of oestrus in the cow.

ARMSTRONG, R. W. and CAMERON, A. E., Hexoestrol implantation of Blackface wether lambs.

BELLIS, D. B., Requirements of bacon and heavy pigs for certain essential amino acids.

BRUMBY, P. J., Relationship of genetic merit to herd differences in milk production.

DICKINSON, A. G., Modification of gestation length in cattle by environment.

DODSWORTH, T. L. and BALL, C., Treatment of beef cattle with tranquillisers and antibiotics.

DUCKWORTH, J. E., COOPER, M. McG., DUNKIN, A. G., TAYLOR, J. M. and HOLMES, W., Progeny testing of bacon pigs at Wye College.

HOLMES, W., JONES, J. G. W. and DRAKE-BRACKMAN, R. M., Feed intake of grazing cattle. II. The influence of size of animal.

KERR, J. A. M., BROWN, W. O. and MORRISON, J., Nutritive value of silage self-fed to fattening cattle.

LODGE, G. A., McDONALD, I. and MACPHERSON, R. M., Relative influence of birth-weight and creep-feed consumption on weights of sucking pigs.

LODGE, G. A., McDONALD, I. and MACPHERSON, R. M., Weight changes in sows during pregnancy and lactation.

PRESTON, T. R., GREENHALGH, I., BOYNE, A. W., CRICHTON, J. A., ROLFE, E. J., BROWN, A. and DODSWORTH, T. L., The effect of hexoestrol on eating quality of beef and lamb.

RENDEL, JAN, and GAHNE, BO., Parentage tests in cattle using erythrocyte and serum transferrins.

WALKER, E. A., and TAYLOR, J. H., Effect of parenterally administered iron upon the growth of piglets.

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Chr. Hansen's Laboratory Enlarges Staff

Chr. Hansen's Laboratory has announced the appointment of DR. R. L. SELLARS as Research Manager for the recently completed Dairy Culture Center. Dr. Sellars, a native of Indiana, received his A.B. Degree from Earlham College, his M.S. in bacteriology and Ph.D. in dairy bacteriology from Purdue University.

R. L. Sellars

Dr. Sellars brings to Chr. Hansen an extensive background in food and dairy bacteriology, with specialized training in bacteriophage and lysogenic lactic organisms. During his training Dr. Sellars was an instructor in food bacteriology, and since graduation in 1957 has been employed as director of quality control for a large Indiana dairy and foods firm.

With the completion of the Culture Research Center, a reorganization of the staff has been made to enable more thorough and extensive research and service to the Dairy Industry. DON EAKLE, Director of the Culture Department, will coordinate research, production, and extension service. Dr. Sellars will be in charge of research. GORDON SANDERS, a veteran of 25 years in dairy laboratory work, will be in charge of production of Dri-Vac lactic cultures and cultured products.

Don Eakle

Gordon Sanders

Foremost Dairies Promotes Parker

R. E. PARKER has been appointed Quality Control Manager at Western Condensing Company, Division of Foremost Dairies, to replace ARA O. CALL, who recently resigned to accept a missionary assignment with the Mormon church.

R. E. Parker

Since joining Western Condensing in 1947, Mr. Parker has worked extensively in various product control and development capacities, including Director of the Central Control Laboratory. He is a graduate of the University of Minnesota, where he specialized in Agricultural Biochemistry. He is an active member of the American Society for Quality Control.

International Luncheon Set for DSI 15th Annual Meeting

Plans for an international luncheon as the high light of Dairy Society International's Fifteenth Annual Meeting on October 22 have been announced by G. W. WEIGOLD, DSI Managing Director. Headquarters will be at the Sheraton-Carlton Hotel in Washington, D. C.

Not only will the meeting point up the round-the-world efforts of the Society and its members to increase the use of the world's milk, but the luncheon menu will be international also—in honor of United Nations Day, which falls on October 24, two days later. DSI, which is a consultant on dairy matters to the UNICEF Board, is one of many nongovernmental organizations observing this day, many of them also serving an around-the-world luncheon or dinner.

The DSI meeting traditionally attracts a number of Embassy guests and it is expected that this meeting, since it is the first in many years to be held in Washington, will be attended by an even more representative cross-section of the diplomatic world. The luncheon is an open one with nonmember industry people invited to participate along with DSI members. The Society has members in 45 to 50 countries. Program details will be announced later.

Symposium on Recent Developments in Research Methods and Instrumentation at NIH

Final plans have been announced for the 11th Annual Instrument Symposium and Research Equipment Exhibit to be held October 9-12 and October 10-13, respectively, at the National Institutes of Health in Bethesda, Maryland. The NIH is the principal research center of the U. S. Public Health Service.

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At the research equipment exhibit, 121 of the nation's leading manufacturers of electronic, mechanical, and optical instruments for laboratory and clinical research will display a wide array of the latest scientific apparatus.

This year's symposium will consist of seven sessions. DR. ALTON MEISTER, chairman of the Department of Biochemistry, Tufts University School of Medicine, will preside over the opening session, dealing with applied gas chromatography.

Other topics on the four-day scientific program are: Factors influencing the interpretation of infrared spectra, optical rotatory dispersion, thermogravimetric analysis, electron probe analysis, the application of physiological instrumentation to clinical problems, and electron magnetic resonance.

Committees to Consider 3-A Sanitary Standards

Ten impending standards, or amendments to existing standards, will be on the agenda when the committees that formulate 3-A Sanitary Standards for Dairy Equipment gather for their next regular semiannual meeting. About 100 engineers, processing technologists, milk and food sanitarians, and federal officials will attend the sessions at the Marriott Key Bridge Motor Hotel in Washington, D. C., October 3-5, 1961.

Three of the ten standards are expected to be completed by the committees at this fall's meeting. They are the Rubber Standards, the Ice Cream Freezer Standards, and the Fillers and Sealers for Single Service Containers Standards.

Seven others to be considered are: Plastics Standards, Automatic Venders Standards, Air-Under-Pressure Standards, Batch Pasteurizer Standards, Separators and Clarifiers Standards, Amendment to Fittings Standards, and Amendment to Pump Standards.

A tour of the Agricultural Research Center at Beltsville, Md., October 2, has been arranged for participants who may arrive early for the sessions, and additionally, an October 5 tour to the National Bureau of Standards and laboratories at the U. S. Department of Agriculture will be available to those interested.

Office of Resource Development Established

The establishment of an Office of Resource Development (Environmental Health) within the Bureau of State Services was announced recently, as another step in the reorganization of the Public Health Service to provide, among other purposes, a more coordinated, integrated, and intensive focus upon the public health problems created by environmental hazards stemming primarily from air and water pollution, exposure to radiation, and occupational and industrial developments.

The Office of Revenue Development, headed by F. A. BUTRICO (Engineer, Director Grade), is a staff arm to DR. R. J. ANDERSON, Assistant Surgeon General and Deputy Chief of the Bureau of State Services.

Completed Theses

M.S. Degree:

DONALD GEORGE EIPP—A consumer preference survey of creamed cottage cheese with pimiento and raisin additives. The University of Georgia.

RICHARD S. SMITH—Influence of milk protein on flavor and physical properties of low fat frozen desserts. Virginia Polytechnic Institute.

ALAN S. TODD—Effects of parathyroid extract on calcium, phosphorus, magnesium, citric acid metabolism in dairy cattle. The University of Georgia.

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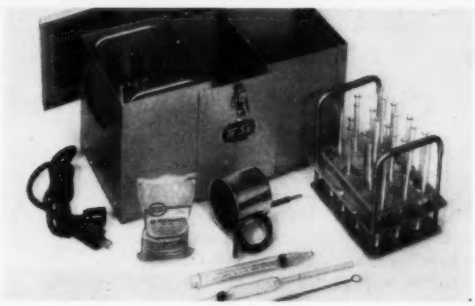

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>
> —Russell Roberts
> Trainee Sealtest Foods
> Southern Division
> Atlanta, Georgia

CONTENTS

SECTION I states the objectives and requirements of the program.

SECTION II contains training schedules for all phases of plant operation for all types of plants. Many questions are asked for the trainee to answer before passing from one phase of training to the next, and progress reports and rating forms are provided.

SECTION III covers management development. Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

The Appendix contains a list of professional and trade organizations and reference reading publications.

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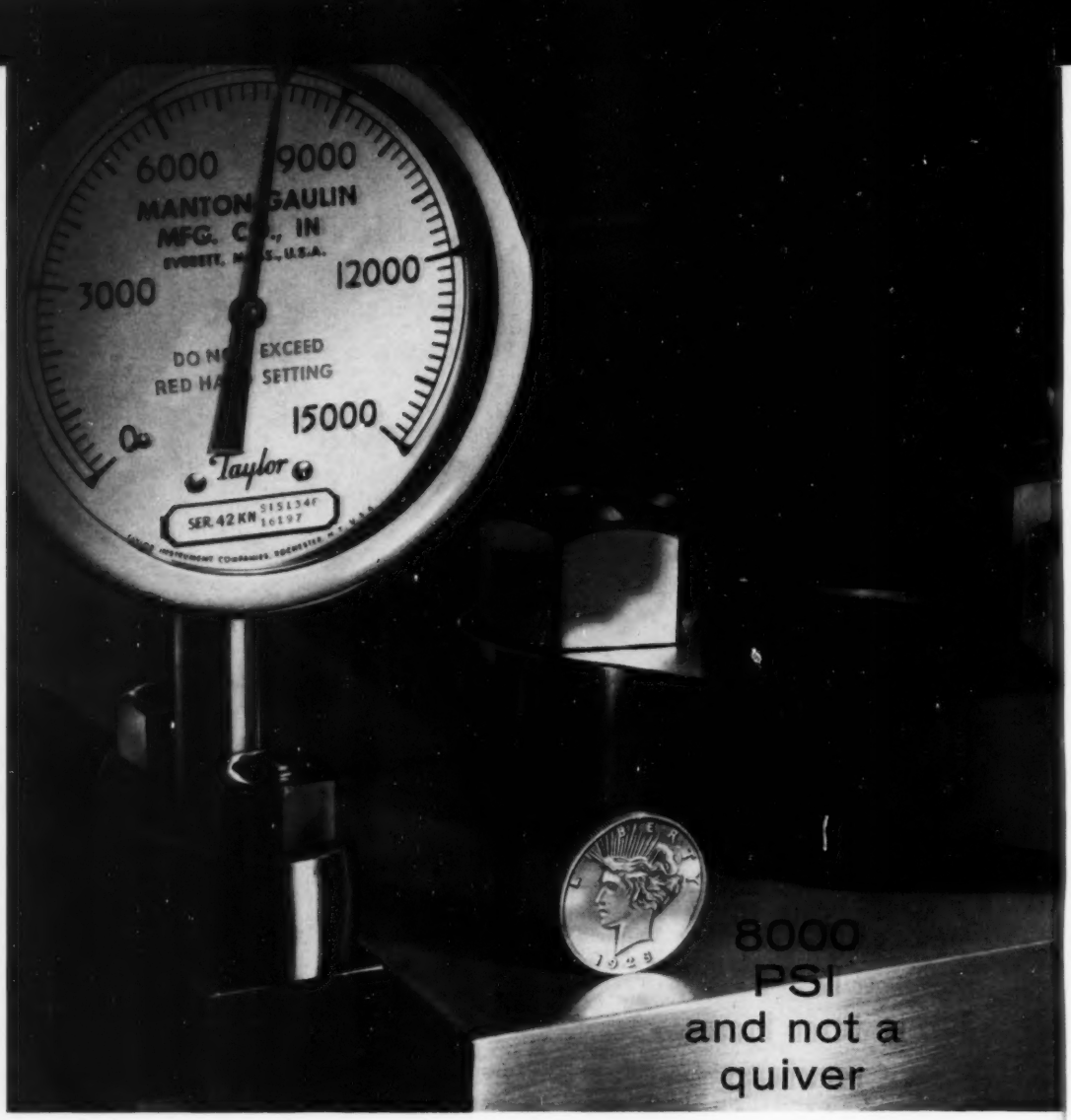

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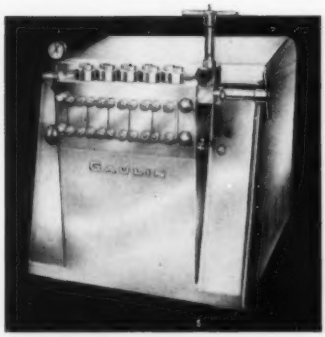

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RESEARCH PAPERS

PRESENCE AND SIGNIFICANCE OF LACTULOSE IN MILK PRODUCTS: A REVIEW[1]

SUSUMU ADACHI[2] AND STUART PATTON
Department of Dairy Science, Pennsylvania Agricultural Experiment Station, University Park

SUMMARY

Lactulose is produced from lactose during the heat processing and storage of certain dairy products. This sugar is somewhat sweeter and more soluble than lactose. It also seems to have unique growth-promoting properties for certain desirable types of Lactobacilli in the intestinal tract of the infant. The chemistry and possible nutritional roles of this sugar are reviewed. The relationship of lactulose to bifidus factor and other carbohydrate moieties of milk also are considered.

It is well known that lactose is the major carbohydrate in the milks of mammals and that other carbohydrates are present at low levels, if at all. Thus, most of the work has concerned lactose. Reviews on manufacture, use, and physiological effects of lactose have been made (22, 65, 136, 137, 138). The presence in milk of carbohydrates other than lactose has become increasingly evident from chromatographic experiments enabling the separation of complex mixtures (42, 56). The most important developments in this field of carbohydrate chemistry have been: the discovery of bifidus factor and the determination of its structure and those of other related oligosaccharides in milk, especially in human milk; the determination of the constituents of the glycomacropeptide released from casein by the action of rennin; the discovery of secondarily formed carbohydrates in milk products and the progress of knowledge on the mechanism of the Maillard (browning) reaction in dairy products.

The present review will cover information on carbohydrates, particularly lactulose, formed secondarily in milk products. Recapitulation of literature on native carbohydrates of milk, however, has been necessary in order to bring the characteristics of lactulose into bold relief.

CARBOHYDRATES OTHER THAN LACTOSE IN MILK

Lactose no longer is considered unique to milk since its discovery in a plant (84). Nor is it considered the only sugar in milk, since other carbohydrates have been detected therein. These carbohydrates are often present in combined form with protein, or lipid or other carbohydrates, although they also are found in milk as free monosaccharides, oligosaccharides, or phosphate esters. The

[1] Authorized for publication February 22, 1961, as Paper No. 2529 in the Journal Series of The Pennsylvania Agricultural Experiment Station.

[2] Permanent address: Department of Animal Products Technology, College of Agriculture, Tohoku University, Sendai, Japan.

monosaccharides and other related compounds occurring in milk are recorded in Table 1, with the corresponding literature references.

Free carbohydrates. Most of the free monosaccharides in milks of most species are galactose and glucose, with the exception of ketoheptose in cow's colostrum (107) and of pentose in kangaroo milk (25). The content of these hexoses, however, is not constant and changes with the period of lactation. To cite one example, the content of glucose in cow's milk averages 13.5 mg. % and is greatest at the peak of lactation and gradually decreases to the end of lactation (134). Neither galactose nor glucose is found in rat's milk (118).

On the other hand, the free oligosaccharides may be considerably different in the milks of various animals. Paper chromatographic investigations by Roberts *et al.* (118) have shown that human milk contains 11 unidentified spots which represent substances of oligosaccharide nature as compared to eight for sow's milk, six for rat's milk, and ten for cow's milk. Most of these have not been isolated in crystalline state or characterized as to chemical structure. The components of seven oligosaccharides in cow's milk, as revealed by Trucco *et al.* (133), are shown in Table 2. Other workers also have found oligosaccharides in various milks. Among these are N-acetyl-0-acetylneuraminolactose in cow's colostrum (77, 78), unidentified reducing disaccharides (113), fucosidolactose (73, 98, 114), lacto-N-tetraose (71, 74, 81, 82), lacto-N-difucotetraose (76), lacto-N-fucopentaose I (74), lacto-N-fucopentaose II (75), lacto-N-difucohexaose I (79), and lacto-N-difucohexaose II (80) in human milk. The chemical structures of most of these have been determined by Kuhn and co-workers (70). György, Zilliken, *et al.* have found that most of them are a growth (bifidus) factor for *Lactobacillus bifidus* var. *Penn.* (17, 41, 45, 46, 47, 119, 140). Since the partial difference in physiological effects of cow's milk and human milk on infants may be explained by differences in activities of such bifidus factors (44, 48), the presence of such oligosaccharides in milk is of particular interest.

The presence of phosphate esters of carbohydrates, namely, α-galactose-1-phosphate, α(?)-glucose-1-phosphate, glucose-6(?)-phosphate (60, 96) and a lactose phosphate, in milk have been reported (96). It seems that these phosphate esters are intermediates in the synthesis of carbohydrates in the mammary gland.

Carbohydrates combined with proteins. Various carbohydrates are present not only in whey proteins but also in casein (3, 34, 66, 68, 95, 116, 121, 128, 129). The most characteristic carbohydrates in these proteins are mainly hexosamines; namely, glucosamine and galactosamine, and neuraminic acid, but other monosaccharides, mannose, galactose and fucose, also have been found. The proteins conjugated with carbohydrates have been generally classified as mucoproteins, mucopolysaccharides, and mucolipids, according to their composition (124). Whether most of the milk proteins should be classified as mucoproteins is somewhat academic. However, the true physiologically active mucoproteins are contained in milk serum or the fat globule membrane (39).

Nitschmann and co-workers have found that a glycomacropeptide is released from casein by the primary action of rennin and that this peptide is degraded

TABLE 1

Monosaccharides and related compounds [a] occurring in milk, either free or combined

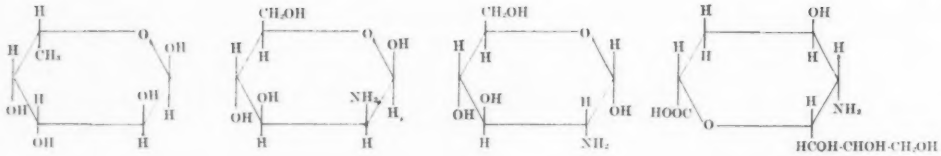
Species	Free [b]	Combined					
		with casein			with whey proteins		with other carbohydrates
		Content % (66)		Constituents	Content % (129)	Constituents	Constituents
Cow	Gal. (14, 57, 94, 118, 123) Glu. (14, 18, 57, 94, 118, 123, 133) Sedoheptulose (107)	Hex. Hxa. Neur.[c]	0.24 0.18 0.39	Gal. (3, 95, 116, 125) Glu. (116) Man. (3, 95, 116) Neur. (130) Glu. A. (3, 95)	Gal. 5% Hxa. 0.5	Gal. (3, 131) Man. (3, 131) Fuc. (131) Neur. (131) Glu. A (3)	Gal. (77, 133) Neur. (77, 133) Glu. (77, 133) Glu. A (133) Man. (133)
Human	Gal. (94, 118) Glu. (94, 118) Unidentified aldohexose (94) Gal., Glu. are not present (118)	Hex. Hxa. Neur.[c]	1.98 1.32 0.76	.....	Glu. A. (120) Gal. A (122)	.....	Fuc. (70, 98, 99, 113) Gal. (70, 98, 99, 113) Glu. (98, 99, 113) Glu. A. (79)
Rat	Gal. (94) Glu. (94)						Gal. (118) Glu. (118)

L-Fucose Galactosamine Glucosamine Neuraminic Acid

[a] Abbreviations: Fuc. (fucose), Gal. (galactose), Glu. (glucose), Hex. (hexose), Hxa. (hexosamine), Man. (mannose), Neur. (neuraminic acid), Gal. A (galactosamine), Glu. A (glucosamine).

[b] Also reported: Gal. and Glu. in sow milk (118), Gal. in opossum milk (43), Pentose in kangaroo milk (25).

[c] Measured as sialic acid.



to neuraminic acid, glucosamine, galactose, and amino acids by hydrolysis with HCl (102, 103). This glycomacropeptide is released from κ-casein, which is thought to be the protective colloid in the calcium-caseinate micelles of milk (101). Thus, the carbohydrate portion of glycomacropeptide may be important in the stability of casein micelles in milk. Work along similar lines has been carried out by Thompson and Brunner (29, 131) and Wake (135).

TABLE 2

The constitution of oligosaccharides in cow's milk

Oligosaccharide	Hydrolysis time	Spots with the same R glucose as the following reference sugars	The activities for *L. bifidus* var. *Penn.*	Spots with the same R Lactose as the carbohydrates in rat's mammary gland
	(min.)			
1	60	Galactose, glucose, mannose, acetyl-glucosamine	Active	
2	60	Galactose, glucose, mannose, acetyl-glucosamine	Active	
3	5	Neuraminic acid, lactose		Compound "E" [a]
4	5	Neuraminic acid, lactose	Inactive	Neuraminolactose [b] (?)
5	60	Lactose, galactose, glucose	Inactive	
6	60	Lactose, galactose, glucose		
7	60	Lactose, galactose, glucose		

[a] Reference (30).
[b] References (132, 142).

Zilliken *et al.* have shown that the bifidus activity of human milk is associated with a mucopolysaccharide-like substance in the nondialyzable fraction of deproteinized milk. They isolated gynaminic acid, namely, N-acetyl-neuraminic acid, with strong bifidus activity from this fraction (140, 141). By polaragraphic investigation of mastitic bovine milk, Breza has established a method for detecting mastitic milk on the basis of increase in the protein double wave (28). Itoh, Adachi, and Nakanishi have further suggested that the in-

crease in this protein double wave is ascribed to a polarographically active mucoprotein which appears to be concerned with protection of tissues against some diseases (64). These experimental results seem sufficient to support the claim that some milk mucoproteins may be physiologically important. It must be emphasized that considerable information is available on the chemical properties of these substances, but that knowledge of their complete structures is still lacking.

THE PRESENCE OF LACTULOSE IN MILK PRODUCTS

The formation of lactulose in milk products. The effects of heat, of prolonged storage, and of fermentation on lactose in milk and milk products have been studied by various investigators. With exception of the fragmentation of lactose, little attention has been paid to the primary stage of transformation in these systems.

In 1953, Honer and Tuckey carried out paper chromatographic studies on reducing sugars other than lactose in raw and heated milk (57). They showed that the spot due to galactose is markedly increased in concentration, but the spot of glucose is faded on paper chromatograms obtained from milk autoclaved at 16 lb. pressure for 15 min., although normal pasteurization did not seem to alter the galactose and glucose pattern in raw milk. In a report on the application of paper chromatography to the study of heat-induced changes in milk, Potter has confirmed the observations made by these workers, and further, has found a considerable number of unidentified compounds reacting with spray reagents used for detecting carbohydrates in highly heated milk (115). Thus, it is reasonable to assume that the increase in concentration of galactose results from the degradation of lactose in the heated milk.

Adachi and Nakanishi also have made approximately the same experiments and have arrived at the same conclusions. Furthermore, they conclusively identified lactulose and tagatose as secondarily formed carbohydrates in heated milk (11, 12, 13, 16). Lactulose was observed to increase considerably during the early stages of heating (14).

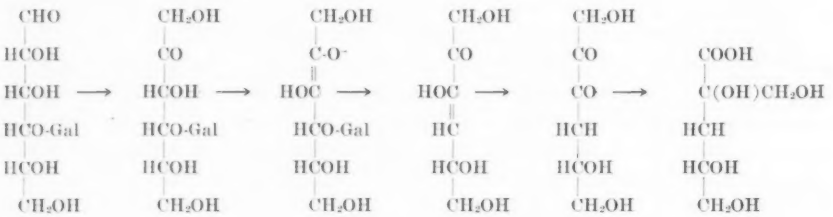
The formation of lactulose in heated milk products has been revealed further by its identification in commercial evaporated milk and deteriorated dried skimmilk. As measured colorimetrically, the amounts of lactulose in 12 lots of evaporated milk were 365 mg. % minimum, 940 mg. % maximum, and 660 mg. % average (8). More recent evidence that lactulose is present in deteriorated dried milk was furnished by Richards and Chandrasekhara's work on the isolation of lactulose, tagatose, and galactose by cellulose column chromatography from dried skimmilk stored at 55° C. in air at 70% relative humidity for 21 days (117). Thus, variable amounts of lactulose may be present in commercial evaporated milk, in dried milk or condensed milk stored under drastic conditions for prolonged periods, and in sterilized milks.

Information on changes in oligosaccharides, other than lactose, and carbohydrate portions of mucoproteins in heated milk is lacking. It may be note-

worthy, however, that the potency of bifidus factor in human milk was not destroyed or altered by autoclaving (46).

The possible mechanisms of formation of lactulose in milk products. The sequences of this transformation reaction in which two atoms of hydrogen in effect, are transferred from Carbon Atom 2 to Carbon Atom 1 of the glucose residue of lactose, giving rise to the fructose residue of lactulose, remains unknown. Although there is no direct evidence to support them, several mechanisms have been proposed.

In 1930, Montgomery and Hudson first prepared lactulose from lactose in lime water by the application of Lobry de Bruyn-Alberda van Elkenstein transformation[3] (97). Corbett and Kenner have further studied the decomposition of lactose by oxygen-free saturated lime water at 25° C. during two to eight days, and have detected lactulose, galactose, tagatose, talose, and saccharic acid in this solution by paper chromatography (32). From the results obtained they have proposed the following mechanism of decomposition of lactose in this solution:



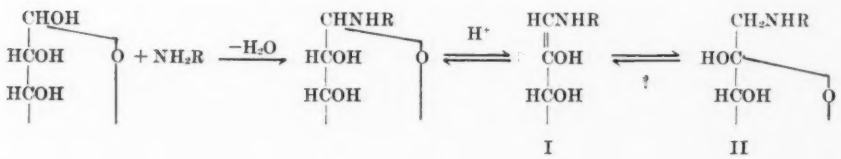
Gal: Galactosyl

In this mechanism it seems clear that the first step in the alkaline lactose solution proceeds by epimerization at C-2 in the glucose residue to the corresponding 2-keto sugar, lactulose, in the manner of the L-A transformation. Hough *et al.* have obtained approximately the same results in experiments on the action of ammonia on lactose (59). From the view that most of the substances identified in heated milk are known to be alkaline fragmentation products of lactose and not to be the fission products derived from an amino-sugar complex, Richards and Chandrasekhara have supported the mechanism postulated by Corbett and Kenner for the decomposition of lactose in dried skimmilk during storage. They have considered that most of the compounds found are formed by the degradation of lactose, namely, L-A transformation, catalyzed by the free basic amino groups of casein, though the Maillard reaction is responsible for the browning of dried milk (117). In fact, the amount of lactulose formed in casein-lactose solutions heated at 120° C. for 2 hr. increased with decreasing hydrogen ion concentration from pH 6.6 to 7.0 (14). These

[3] Abbreviated hereinafter as the L-A transformation.

findings support the view that lactulose at least in part is derived from lactose in the milk systems in accordance with L-A transformation.

On the other hand, in the Maillard reaction between amino acids or proteins and reducing sugars the formation of amino-sugar complex, namely, the formation of N-glycosidic substance and its Amadori rearrangement product, has been considered to be one of the main pathways of the decomposition of carbohydrates (2, 37, 54, 55, 91, 104, 105). The first step is the elimination of water from the reducing group of the carbohydrate and amino group of the amino compound, giving a Schiff's base which in turn forms a 1-amino-1-deoxy-2-ketose, Amadori rearrangement product. The 1,2-enol form I may undergo a dehydration reaction to 5-hydroxymethylfurfural and fragmentation reactions to sugar fission products. In most cases, it is not easy to form fission products from Amadori rearrangement product II, since it is quite stable. Although much evidence supporting the binding of lactose to milk proteins in heated or stored milk systems has been obtained (36, 52, 93, 106, 124), there is little information on the nature of this bond. On the evidence of amino acid-N-glycosides from the tryptic hydrolysates of evaporated milk (3, 4, 5, 6, 7), from other browned foods and biological material (1, 19, 20, 21, 26) and the data of Lea *et al.*, showing the decrease in amino groups of basic amino acids in casein and the increase in the binding of sugars in casein-reducing sugar systems under the dry state (49, 85, 86, 87, 89, 90), it seems conceivable that the bond may be N-glycosidic. It has been well established that carbohydrates generally are not recoverable from the acid hydrolysates of Amadori rearrangement products of amino-sugar complexes. In addition, it has been reported that the Schiff's base of lactose in a lactose-valine system is unable to undergo an Amadori rearrangement (50). In spite of these results, however, it seems possible that lactulose is at least partially derived from N-glycosidic substance in a milk protein-lactose complex, since a p-tolyl-N-lactosylamine has been recently shown



to rearrange readily to p-tolyl-N-isolactosylamine, its Amadori rearrangement product, and lactulose has been identified from the acid hydrolysates of these compounds (9, 10). More recently, Kuhn and Krüger have reported that p-tolyl-N-lactosylamine undergoes an Amadori rearrangement (83).

Although there is, at the moment, little direct evidence supporting the foregoing postulated mechanisms of lactulose formation in milk systems, it seems probable that two main paths should exist since the pH range of milk systems permits the possibilities of transformation in two ways. The first path is the

L-A transformation. This is in essence an alkaline isomerization, but it has been known that alkaline media are not always required but Brönsted base and its conjugated acid are essentially necessary. Therefore, it is not surprising that this transformation has been carried out even under neutral or acidic conditions which could prevail in milk and milk products. For example, McIlvaine's citrate-phosphate buffer at pH 2.21 and 6.95 and potassium diphosphate at pH 6.4 to 6.6 have been used for the isomerization of aldoses to corresponding ketoses by Petuely (108) and Englis and Hannan (38), respectively. Furthermore, Kass and Palmer have suggested that an appreciable, but constant, conversion of lactose to ketose, or substances not oxidized by sodium hypoiodite, may occur in lactose solution heated with various buffers at pH 1.85 to 8.05, though they did not identify the secondarily formed ketose (67). Thus, it seems most reasonable to assume that the presence of phosphate, citrate, and milk proteins as ampholytes in milk is favorable to the formation of lactulose from lactose through L-A transformation, in spite of neutral or slightly acidic conditions. Such formation of lactulose in milk systems would follow the sequence of reactions, through the hybrid anion, through the enediol and finally, through the hybrid anion, essentially as postulated by Speck for the mechanism of aldose-ketose isomerization (126).

The second theory of the mechanism of lactulose formation assumes the formation of lactosylamine which undergoes Amadori rearrangement to lactulose by hydrolytic degradation. It is to be expected that the presence of citrate and phosphate or milk proteins as ampholytes and formation of organic acids in milk products results in a faster transformation of lactose to lactulose through Amadori rearrangement of lactosylamine, since the presence of an active proton donor facilitates the Amadori rearrangement (55).

Thus, these findings on the mechanisms of lactulose formation in milk systems may be represented, as shown in Figure 1.

POSSIBLE USES OF LACTULOSE IN MILK PRODUCTS

Chemical properties of lactulose. Lactulose shows the composition and molecular weight of a disaccharide $C_{12}H_{22}O_{11}$. It gives the Seliwanoff test and Dische-Borenfreund test for ketoses (35), reduces Fehling's solution on heating, is not oxidized by hypoiodite or bromine, and gives fructose and galactose by hydrolysis with 1% sulfuric acid at 100° C. for 8 hr. Its anhydrosazone melts at 226° C. with decomposition, shows $(\alpha)_D^{20} - 174$, and is identical with lactose anhydrosazone. In aqueous solution at 22° C. its $(\alpha)_D$ value is −23.9 in 2 min. after solution and the final value is −51.5. The structure of lactulose is shown to be 4-0-β-D-galactopyranosyl-D-fructose and the crystalline sugar is an alpha form. Further, the fact that the ring structure of the fructose component is furanose has been confirmed by the work of Isbell and Pigman (62). Lactulose is hygroscopic and very soluble in water. It is recrystallized from 50% methanol in hexagonal, colorless plates often grouped in clusters and melts at 158° C. (97) or 163° C. (27) or 163-165° C. (87). The octaacetate of lactulose is prepared

by low-temperature or room-temperature acetylation with a mixture of acetic anhydride and pyridine (27, 40). This derivative melts at 138-139° C. and shows $(\alpha)_D^{20} - 6.6$ in chloroform ($c = 4$). The infrared spectrum of lactulose octaacetate shows strong absorption bands in the regions characteristic of the carbonyl group, methyl group, acetyl group and a characteristic band at 9.8 μ which is absent in the spectrum of lactose octaacetate (63).

Isbell was the first to study the action of alkali on lactulose and he has suggested that the increased dextro rotary power in lime water may be attributed solely to partial reversion of lactulose to lactose (61). More recently, Corbett and Kenner have investigated in detail the action of saturated lime water on lactulose at 25° C. They have detected lactose, galactose, tagatose, and saccharic acid by paper chromatography and have observed that the degradation of lactulose proceeds more rapidly than of lactose (33). Corbett further has described a quantitative chromatographic method for separation of the mixture, galactose and lactulose (31).

Synthesis of lactulose. (A) By the Lobry de Bruyn-Alberda van Ekenstein transformation of lactose. Montgomery and Hudson reported that a solution of lactose in saturated lime water at 35° C. for 36 hr. gives a new disaccharide ketose that results from the L-A transformation. They have shown that the sugar can be isolated in crystalline state from the mixture of sugars by oxidizing the mixed aldoses with bromine water in the presence of barium benzoate and they gave the name, lactulose, to this new crystalline ketose (24, 97). Lactulose by this method, however, is often contaminated by trace amounts of other carbohydrates. This preparative technique has since been improved. It is recommended that the lime water reaction products be treated with an anion exchange resin in the $-HSO_3$ form to remove aldoses (11). Direct acetylation of lactulose has been suggested (27) in order to remove contaminants. Bots has prepared lactulose octaacetate in good quality by acetylation (acetic anhydride and pyridine at room temperature) of the crude reaction product obtained by treating lactose with lime water and removal of part of the unreacted lactose by crystallization (29). He then obtained lactulose from the octaacetate by heating with 0.1 *N* HCl for 24 hr. at 71° C. The yield from the acetate was 40 to 50%.

(B) By Amadori rearrangement of p-tolyl-N-lactosylamine. As shown previously, it has been found that lactulose is formed by the action of HCl on p-tolyl-N-lactosylamine and p-tolyl-N-isolactosylamine. Adachi has prepared these lactosylamine compounds by treating lactose with p-toluidine (9, 10). Kuhn and Krüger have reported that lactulose can be prepared in good yield from 1,3-di(p-tolyl)-3-lactosyl-triazine by decomposition with a mixture of hydrochloric and hydroiodic acids and by catalytic hydrogenation of the decomposition product, followed by deamination of 1-deoxy-1-amino-lactulose with $NaNO_2$ to give lactulose. The total yield from lactose was 17% (83).

(C) By reduction of lactosone. Lactosone (4-0-β-D-Galactosyl-D-arabinohexosone) is reduced to lactulose by treating with zinc dust in acetic acid solu-

tion (16). This method, however, may be impractical unless the preparative method of lactosone is further improved regarding yield.

Biochemical and physiological properties of lactulose. Little is known concerning the biochemistry of lactulose, but since there are chemical similarities between this substance and lactose, it would seem that there might be some

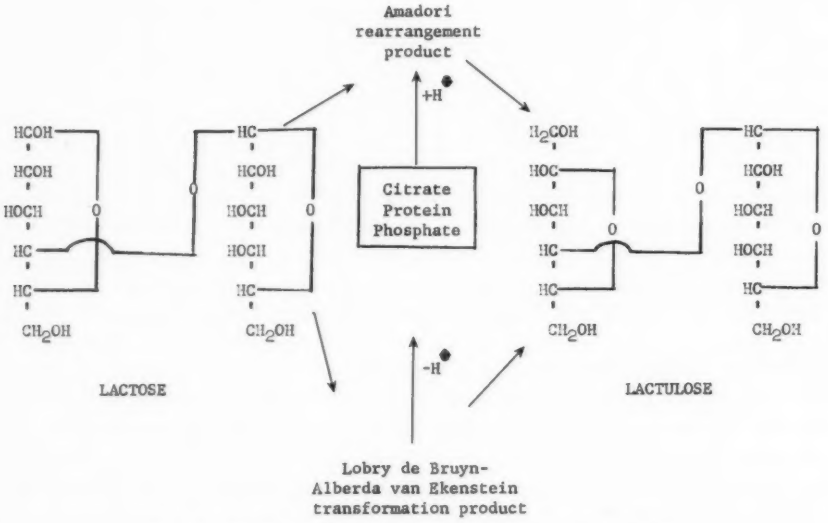


FIG. 1. The mechanisms of lactulose formation in milk.

relationship between their biochemical properties. A study by Helferich and Pigman indicated that lactulose is cleaved by sweet almond emulsin the same as is lactose (51). Lactulose is hydrolyzed more easily by this enzyme than lactose and a number of its derivatives with the exception of phenyl lactoside (112). However, knowledge of the action of carbohydrases in the intestine, gastric juice, and saliva on lactulose is lacking.

The effect of lactulose on *Lactobacillus bifidus*, a unique predominant microorganism in the intestinal flora of the breast-fed infant, has been studied with possible use of the sugar in infant foods in mind. Petuely has shown that a pure culture of bifidus flora is produced in the infant, and in older children as well, when lactulose is added in an amount of approximately 1.2 g. per 70 cal. daily in a diet the lactose content of which is at least 2.5 times as large as the protein content (109, 110). Without lactulose such a proliferation of the bifidus flora did not occur. MacGillivray *et al.* have found that the lactobacilliary response in the intestine reached at least 90% when a lactulose supplemented milk of the following composition was fed to infants: 2.25% protein, 2.4% fat, 6% lactose, and 1% lactulose. They also observed favorable effects on the characteristics of the stools with this milk. However, they did not attempt to

demonstrate the presence of *L. bifidus* as such, and the diet did not show any value for eliminating *Escherichia coli* from the intestine (92). Hirata has studied the effects of lactose and lactulose on the microbial flora in the digestive tract of the infant with in vivo experiments, using a special catheter to deliver the carbohydrates to the upper part of the intestine without their suffering the action of gastric juice. He found that the bifidus flora in the stool of the infant was remarkably increased when lactulose was present (53). Although the mechanism of lactulose utilization in vivo is not yet firmly established, it seems reasonable to conclude that this carbohydrate plays an important role in promoting growth of *L. bifidus* in infants.

In bifidus factor, as defined by the works of György, Kuhn, and their associates, glucosamine occurs in common and appears essential in the various biologically active moieties. However, lactulose does not contain glucosamine, and thus it has been proposed by Petuely as a different bifidus factor. Whether some type of conversion under physiological conditions accounts for this discrepancy is not known. In addition, the bifidus factor of György *et al.* is not necessary for the growth of many strains of *L. bifidus*.

Use of lactulose in milk products. Beyond the properties already described, the merits of lactulose in milk manufacturing are high solubility in water and sweetness. It is sweeter than lactose but not as sweet as sucrose and does not crystallize easily even in concentrated solutions. Thus, lactulose might be used practically as a substitute for lactose or sucrose in milk powder manufacturing to improve the solubility of the products; and in ice cream making and condensed milk manufacturing to prevent sandiness, if a completely satisfactory preparative method for lactulose can be established.

In recent years, Petuely has proposed a lactose preparation that induces the growth of substantially pure bifidus flora in the digestive tract of the infant (111). This product was prepared by heating a lactose solution at a fairly high temperature, but below 150° C., in a pH range of 5 to 9. The essentially lactose-free concentrate finally obtained was used by adding it to food preparations such as milk so that a lactose-protein quotient of 2.6 is secured. Koyama recently has prepared a similar product from rennet whey by prolonged heating under strongly alkaline condition (69). He has reported that the gain in body weight, decrease in pH of the stools, and increase in bifidus flora of infants taking milk containing this product are remarkable. Yasaki and Kondo have studied the effect of feeding infants milk powder mixed with whey processed by Koyama's method (139). As shown in Table 3, decided increases in bifidus flora in the stools were produced by feeding milk powder containing this processed whey. In addition, better body growth, tendency toward excreting of soft stool, decreases in pH, increases in excreting times and of yellow coloring of stools, and large increases in red blood cells were observed in the groups taking the experimental samples. Since these physiological characteristics are ordinarily obtained in infants nurtured by human milk, Yasaki and Kondo have suggested that milk powder may be humanized considerably

by the addition of processed whey. Thus, it seems probable that the active factor of the product for bifidus flora in infants may derive principally from the lactulose which it contains. However, since the content and physiological properties of tagatose, and possibly other heat-generated substances, in these products are not known, lactulose may not fully account for the results obtained. Of interest in this connection, Auclair and Portman have observed a stimulating effect on *Lactobacillus lactis* by the formic acid produced when milk is autoclaved (23).

TABLE 3

Bifidogenic response [a] in infants produced by processed whey

		Milk powder + processed whey	Control milk powder
Before experiment		4.8	5.0
Experimental periods	1st week	41.6	5.1
	2nd week	34.0	10.4
	3rd week	28.9	4.8
	4th week	34.0	5.1
After experiment		8.6	5.3

[a] Percentage of bifidus flora to total number of microorganisms in stool.

Concerning keeping quality, the dry milk products of Yasaki and Kondo are slightly brown in color and are very hygroscopic, but little information on storage stability is available. Many interesting research problems concerning the effect of lactulose or lactulose preparations on the chemical, biochemical, and physiological properties of milk products await exploration by dairy scientists.

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COMPOSITION AND BACTERIAL GROWTH AS FACTORS AFFECTING THE BODY OF PASTEURIZED PROCESS CHEESE SPREAD [1]

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SUMMARY

Effects of variations of composition on the firmness of pasteurized process cheese spreads made with Dariworld cheese were determined by (1) modifying moisture and fat contents of a base formula and (2) by substituting nonfat dry milk (NDM), lactose, or dried whey for part of the solids-not-fat supplied by cheese (CSNF). Firmness of spreads varied inversely with moisture, where the proportion of solids-not-fat to fat was held constant. Firmness was not affected by changes in fat when the ratio of moisture to solids-not-fat was not altered. Use of increased amounts of lactose and dried whey decreased the firmness of spreads. Addition of NDM was less effective in decreasing firmness. Firmness increased when the proportion of moisture to cheese solids-not-fat (H_2O/CSNF) was decreased; this relationship was notably regular for all modifications.

Some samples containing added lactose and NDM increased markedly in firmness and acidity during 90 days of storage at 90° F.; gram-positive bacilli were found in large numbers in all batches of such firm-bodied spreads. Spreads without added NDM or lactose did not increase in firmness during storage for 90 days at 90° F., regardless of levels of moisture or fat.

The firmness of pasteurized process cheese is affected by the amount and proportions of constituents such as moisture, and the casein/fat ratio of the cheese used in its manufacture (14, 17-19). The fat content and the ratio of casein to moisture of process cheese are important factors influencing smoothness of consistency (15).

Certain optional ingredients in cheese spreads may affect their firmness. Sommer and Templeton observed that dried whey did not contribute significantly to firmness, but that nonfat dry milk increased firmness so that butter or cream had to be added to attain spreading qualities (16). Their observations were based upon cheese spreads containing approximately 44% moisture, 26% fat, and 46% fat in the dry matter.

Firmness of body of process cheese and process cheese spreads may be accounted for in part by interrelationships of physical properties imparted by varying amounts and proportions of cheese, water, cream, butter, dried whey, and nonfat dry milk used in their manufacture. Effects of individual additives may be concealed by the resulting dilution or variation of proportions of other components. This study was undertaken to evaluate effects of variations of some of the individual components of cheese spreads. Variations of kinds and amounts of emulsifiers were not included in this study.

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METHODS

Experimental design. A base spread and 11 modifications of it were used in this study. The 11 modifications were produced by altering the base formula shown in Table 1 as follows: (1) decrease of 3% moisture, (2) increase of 3% moisture, (3) increase of 6% moisture, (4) increase of 3% fat, (5) increase of 6% fat, (6) replacement of 3% of the solids-not-fat supplied by the cheese (cheese solids-not-fat or CSNF) with nonfat dry milk (NDM), (7) replacement of 6% of CSNF with NDM, (8) replacement of 3% of CSNF with lactose, (9) replacement of 6% of CSNF with lactose, (10) replacement of 3% of CSNF with dried whey, and (11) replacement of 6% of CSNF with dried whey.

TABLE 1

Calculated composition of base spread

	Total	Fat	Cheese SNF[a]	Noncheese SNF[a]	Moisture
			(%)		
Dariworld	75.2	19.2	23.0	0.0	33.0
Butter	1.2	1.0	0.0	0.0	0.2
Disodium phosphate	2.5	0.0	0.0	2.0	0.5
Salt	0.6	0.0	0.0	0.6	0.0
Lactic acid	0.4	0.0	0.0	0.3	0.1
Added water	5.2	0.0	0.0	0.0	5.2
Steam condensate	14.9	0.0	0.0	0.0	14.9
Totals	100.0	20.2	23.0	2.9	53.9

[a] Solids-not-fat.

Formulation of spreads. The proportions of ingredients other than the one to be varied were kept the same in the modification as in the base spread itself. Thus, when moisture was altered, the proportion of fat to solids-not-fat was kept constant. In the same way, when fat was varied, the proportion of moisture to solids-not-fat was kept constant. Such adjustments were made by varying the amount of cheese, butter, or water.

Manufacture of cheese. Dariworld, a semisoft ripened cheese, was made especially to contain 45% fat in the dry matter, so that it could provide 100% of the protein and almost the entire amount of the fat in the base spread (10). The cheese was ripened at 50° F. for 75 days before processing.

Manufacture of spread. All spreads were processed in 10-lb. batches. Cheese, disodium phosphate, sodium chloride, butter, water, and any other necessary ingredients, like NDM, dried whey, or lactose, were added together in a modified Hobart mixer (13). The mixture was heated to 140° F. by direct injection of clarified steam, and at that temperature enough lactic acid was added to give a pH of 5.49 ± 0.1 in the finished spread. Heating was continued until the temperature reached 185° F. The spread was held at this temperature for 1 min., homogenized at 1,500 lb. pressure, and pumped into 8-oz. jars.

Examination of spreads. Forty-eight hours after processing, the spreads were tested for firmness at 70° F. by the penetrometer method (13). (Penetrometer

values are related inversely to spread firmness.) Milk fat was determined by the Babcock method. The A.O.A.C. vacuum drying procedure was used to determine the moisture content of the spreads (1). The pH was determined by means of the quinhydrone electrode (20).

Storage of spreads. Spreads were stored at 70 and 90° F., to test their stability under normal and undesirable conditions of holding. Samples were removed from storage after 15, 30, 60, and 90 days and tested for firmness and pH at 70° F.

Microscopic examination. Direct microscopic cell counts of spreads were made after 90 days of storage at 70 and 90° F., using the methods of Foster and Frazier (7, 8) and Hucker's modification of the Gram stain (2). Spore stains were made by Bartholomew and Mittwer's cold method (3).

RESULTS

Effect of moisture on firmness. Analyses of spreads with adjusted moisture contents are shown in Table 2. The differences between the actual and calculated percentages of moisture of the adjusted lots were probably caused by unavoidable variations in the moisture content of injected steam. Adjustments of the moisture content of the base spread gave penetrometer values ranging from 45 in the sample with the lowest moisture to 179 penetrometer units in

TABLE 2

Means and standard deviations of analyses of the base spread and its 11 modifications. Values are means of three lots, except for base spread, which is the mean of six lots

Modification [a]	Moisture	Fat	Solids-not-fat	Cheese solids-not-fat [b]
	(%)			
Base (calculated composition)	53.9	20.2	25.9	23.0
Base	53.4 ± .77	19.8 ± .62	26.9 ± .39	23.9 ± .35
	Moisture adjusted			
Base − 3% H_2O	49.7 ± 1.53	21.3 ± .61	29.0 ± .93	26.0 ± 1.84
Base + 3% H_2O	55.1 ± .42	19.1 ± .17	25.8 ± .49	22.7 ± .58
Base + 6% H_2O	58.2 ± .16	18.5 ± .31	23.3 ± .44	20.3 ± .38
	Fat adjusted			
Base + 3% fat	49.8 ± 1.36	24.1 ± .45	26.1 ± 1.01	23.1 ± .89
Base + 6% fat	47.9 ± .91	26.9 ± 1.01	25.2 ± .36	22.2 ± .31
	NDM substituted for CSNF			
3% NDM	52.2 ± .19	20.1 ± .47	27.7 ± .60	23.0 ± .51
6% NDM	53.3 ± .53	19.9 ± .40	26.9 ± .67	20.7 ± .51
	Lactose substituted for CSNF			
3% lactose	53.1 ± .57	20.1 ± .41	26.9 ± .20	20.7 ± .16
6% lactose	53.5 ± .96	20.2 ± .49	26.3 ± .61	17.2 ± .41
	Dried whey substituted for CSNF			
3% dried whey	53.3 ± .38	19.4 ± .45	27.3 ± .20	22.0 ± .16
6% dried whey	53.5 ± .32	19.7 ± .44	26.8 ± .38	19.3 ± .28

[a] Percentages are based on weight of finished cheese spread.

[b] Fifty per cent of the weight of added NDM and 28% of the weight of added dried whey are included in the weight of the cheese solids-not-fat.

the sample with the highest moisture content (Table 3). The differences in these penetrometer values were highly significant ($P = 0.01$).

Effect of fat on firmness. Increasing the fat even to a level of 6% above that of the base caused only slight variations in penetrometer values. These differences, which are shown in Table 3, were not significant statistically.

pH of spreads. The mean pH values of all spreads 24 hr. after making, shown in Table 3, varied from 5.45 to 5.53. Individual pH values not shown in Table 3 ranged only between 5.40 and 5.56. These minor differences in pH would not be expected to affect the firmness of the spreads significantly (12).

Effects of proportions of moisture, solids-not-fat, and fat on firmness. The data of Table 3 show that adjustments of moisture which gave 1.71 to 2.50 lb. of moisture per pound of solids-not-fat (H_2O/SNF) in the first three modifications were associated with penetrometer values ranging from 45 to 179. At the same time this adjustment of moisture caused the ratio of moisture to fat (H_2O/F) to vary from 2.33 to 3.16 as shown in Table 3. Extra fat was added to the base spread to give a ratio of H_2O/F of 2.07 in the series with 3% added fat and 1.78 in the second series in which the fat of the base spread was increased 6%; H_2O/SNF was 1.91 in the first and 1.90 in the second series. Penetrometer values were not altered significantly by these adjustments of fat. The results suggest that firmness depends upon the ratio of moisture to solids-not-fat in the spread. This assumption is substantiated in Figure 1, in which the smooth curve depicts the relationship between penetrometer values and H_2O/SNF. The relationship between these two factors was almost linear above the sharp break in the curve which occurred just below H_2O/SNF ratios of 2.0 to 2.1. The effect on spread firmness caused by unit changes in H_2O/SNF was six times greater above this break than below it.

In a paper published after this study was completed, Jakubowski and Bijok (11) reported a sharp change in the relationship between moisture and firmness in processed Tilsit cheese when the moisture was increased above 55% with 40% fat in the dry matter. Our calculations show that the H_2O/SNF ratio at this point approximated 2.1.

Effect of NDM, lactose, and dried whey on firmness. Penetrometer values were increased when NDM, lactose, and dried whey were added, and were approximately doubled when maximum amounts of these ingredients were used (Table 3). Percentages of moisture, fat, and SNF shown in Table 2 or H_2O/SNF and H_2O/F ratios in Table 3 remained relatively constant in these modifications. However, percentages of cheese solids-not-fat in Table 2 appeared to vary inversely with penetrometer values.

Ratios of H_2O/CSNF were calculated for the base and spreads which varied in moisture and fat (Table 3). The shape of the curve drawn in Figure 2 to fit these ratios plotted against their penetrometer values duplicated the curve in Figure 1. Spreads in which 3% of CSNF were replaced by lactose gave values of H_2O/CSNF and penetrometer values which coincided with the curve in Figure 2. Substitutions of lactose for 6% of CSNF in the base produced somewhat lower penetrometer values than those predicted by the curve. The

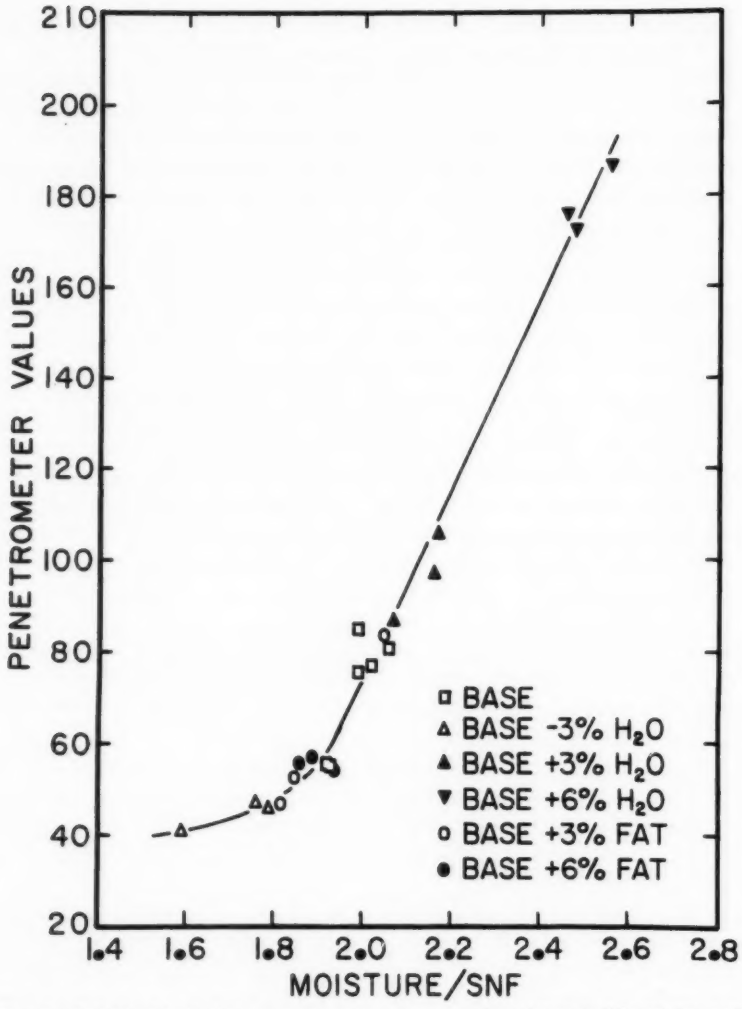

FIG. 1. Relationship between penetrometer values and moisture/solids-not-fat ratios of the base spread and its modifications which varied in moisture and fat.

water phase of this spread contained 11.2% lactose, or nearly a saturated solution at the 70° F. temperature at which the tests for firmness were made. The viscosity associated with this concentrated solution of lactose may be responsible, at least in part, for the slightly greater firmness than that predicted by H_2O/CSNF.

Since lactose at the 3% level of addition did not affect the relationship between H_2O/CSNF and firmness, it was assumed that lactose in NDM and dried

whey would tend to react on firmness as pure lactose had done. Therefore, H_2O/CSNF ratios were recalculated for these two modifications to include only the nonlactose portion of NDM and dried whey with the cheese solids-not-fat. It was assumed in making these calculations that the nonlactose portions equaled 50% of the NDM and 28% of the dried whey. These modified ratios plotted against penetrometer values for spreads containing NDM fell very close to the curve in Figure 2. Points plotted for spreads containing added dried whey fell above the curve. This effect may be caused by a lesser degree of hydration or swelling of the whey proteins during processing, or greater fragility of these proteins in the structural arrangement of spreads.

TABLE 3

Means and standard deviations of ratios of certain components, pH, and penetrometer values of the base spread and its modifications

Modification [a]	Penetrometer [b]	H_2O/SNF	H_2O/F	H_2O/CSNF	pH [b]
	(0.1 mm.)				
Base	71 ± 13.0	1.99 ± .08	2.71 ± .12	2.23 ± .06	5.45 ± .05
		Moisture adjusted			
Base − 3% H_2O	45 ± 3.2	1.71 ± .11	2.33 ± .14	1.91 ± .12	5.48 ± .06
Base + 3% H_2O	96 ± 10.6	2.13 ± .06	2.88 ± .03	2.42 ± .06	5.47 ± .03
Base + 6% H_2O	179 ± 7.6	2.50 ± .05	3.16 ± .05	2.87 ± .06	5.53 ± .03
		Fat adjusted			
Base + 3% fat	61 ± 19.9	1.91 ± .13	2.07 ± .10	2.16 ± .14	5.49 ± .08
Base + 6% fat	56 ± 1.2	1.90 ± .04	1.78 ± .10	2.16 ± .05	5.48 ± .04
		NDM substituted for CSNF			
3% NDM	74 ± 1.9	1.89 ± .04	2.59 ± .06	2.27 ± .05	5.47 ± .06
6% NDM	124 ± 14.0	1.99 ± .07	2.68 ± .06	2.57 ± .09	5.52 ± .01
		Lactose substituted for CSNF			
3% lactose	114 ± 11.5	1.97 ± .03	2.64 ± .08	2.56 ± .05	5.48 ± .03
6% lactose	181 ± 10.7	2.04 ± .08	2.66 ± .11	3.11 ± .12	5.50 ± .04
		Dried whey substituted for CSNF			
3% dried whey	114 ± 10.9	1.96 ± .02	2.75 ± .09	2.43 ± .02	5.48 ± .02
6% dried whey	185 ± 10.9	2.00 ± .03	2.72 ± .07	2.77 ± .05	5.49 ± .03

[a] Percentages are based on weight of finished cheese spread.
[b] Penetrometer and pH measurements were made at 70° F., 48 hr. after processing.

Changes in spreads during 70° storage. Penetrometer values of spreads stored at 70° F. decreased very little, only zero to nine units, during the 90-day storage period, excepting those in which equivalent amounts of cheese solids-not-fat had been replaced with 6% nonfat dry milk or 3 and 6% of lactose (Table 4). These three modifications listed in the same order showed decreases in penetrometer readings of 28, 21, and 11 units, respectively. Since experienced judges were able to distinguish between spreads differing by 16.5 units (13), these changes probably would not be of any practical significance, especially in view of the softness of these spreads, which ranged from 114 to 181 units. These changes occurred entirely during the 3rd mo. of storage.

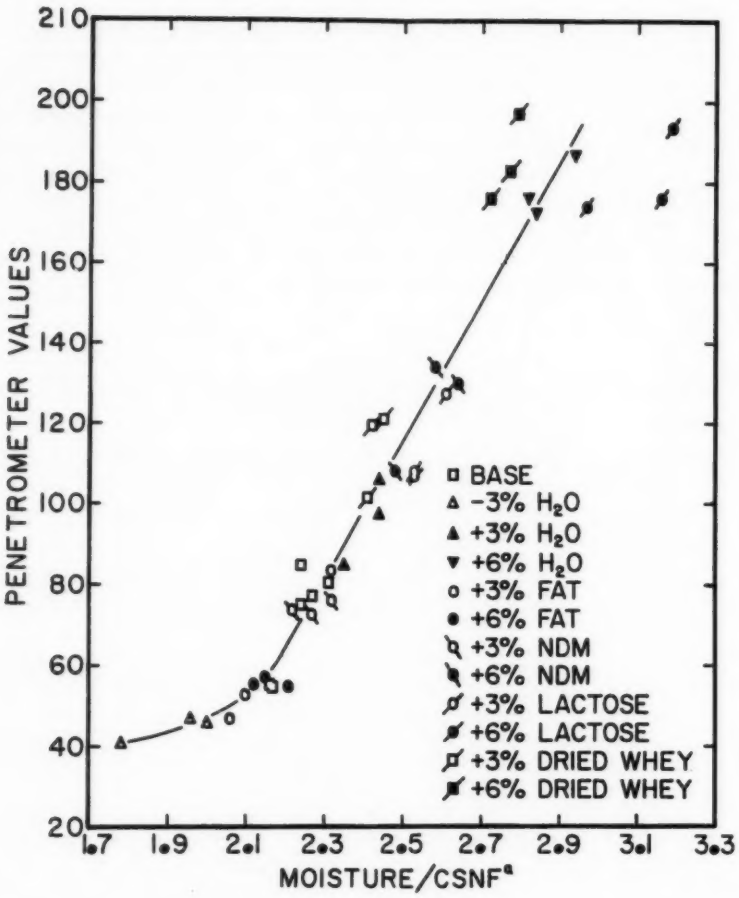

FIG. 2. Relationship between penetrometer values and moisture/cheese solids-not-fat (CSNF) ratios of the base spread and its 11 modifications.

Values of pH of the spreads held at 70° F. remained substantially unchanged during the 90-day storage period.

Changes in spreads during 90° storage. Three of the modifications of the base spread stored for 90 days at 90° F. showed distinct increases in firmness. Tables 4 and 5 show that changes of commercial significance ranging from 57 to 102 penetrometer units occurred in those lots in which 6% nonfat dry milk and 3% and 6% lactose had been substituted for equivalent amounts of cheese solids-not-fat. Decreases in pH occurred in the spreads stored at 90° F. The

[a] Fifty per cent of the weight of added NDM and 28% of the weight of added dried whey are included in the weight of CSNF.

greatest changes of 0.21 to 0.59 pH unit occurred in those lots containing the nonfat dry milk and lactose (Table 5). Spreads containing dried whey were not stored in this study, but there is no reason to believe that they would not react the same as spreads containing NDM or lactose.

Microscopic examination of the spreads revealed the presence of gram-positive cocci and gram-positive spore-forming bacilli in certain batches of cheese. No consistent relationship was observed between numbers of cocci and the excessive firmness of some of the spreads. However, 10 to 49 millions of bacilli per gram of cheese were found in spreads containing the nonfat dry milk and lactose (Table 5). In spreads without these additives the counts of bacilli averaged from 0 to 1.9 million; these spreads included the base spread and those with varied amounts of water and fat. Gram-positive bacilli could not be detected in any of the spreads stored at 70° F.

TABLE 4

Mean penetrometer and pH values of spreads after storage at 70 and 90° F. for 30 and 90 days. Each value is the mean of three lots

	Penetrometer values				pH values			
	70° F.		90° F.		70° F.		90° F.	
Modification [a]	30 days	90 days	30 days	90 days	30 days	90 days	30 days	90 days
	(0.1 mm.)				*(pH)*			
Base	57	67	50	44	5.52	5.47	5.45	5.36
	Moisture adjusted							
Base − 3% H_2O	49	41	44	35	5.51	5.47	5.45	5.36
Base + 3% H_2O	92	94	94	80	5.47	5.50	5.48	5.40
Base + 6% H_2O	180	175	180	183	5.49	5.50	5.49	5.47
	Fat adjusted							
Base + 3% fat	54	52	52	51	5.55	5.50	5.47	5.43
Base + 6% fat	64	50	51	52	5.57	5.51	5.50	5.35
	NDM substituted for CSNF							
3% NDM	75	70	69	58	5.52	5.51	5.45	5.26
6% NDM	108	96	93	67	5.48	5.50	5.42	5.31
	Lactose substituted for CSNF							
3% Lactose	110	93	102	44	5.52	5.46	5.44	5.08
6% Lactose	181	170	167	79	5.51	5.48	5.48	4.91

[a] Percentages are based on weight of finished cheese spread.

It is impossible to explain with certainty from the data in Table 5 why spreads containing 3% NDM did not show the same decrease in penetrometer readings during storage at 90° F. as those containing 6% NDM, 3% lactose, and 6% lactose. Spreads with 3% NDM were firmer than the others when freshly made; hence, a slight change (21%) in the original firmness of these spreads reduced their mean penetrometer reading to 58. This is in the zone of firmness reached by spreads containing higher levels of lactose, which showed decreases of 46 to 61% of their original penetrometer readings during storage. The mean pH of 5.26 of spreads with 3% NDM closely approximated the pH

of spreads with 6% NDM and is well below the pH values of the lactose-poor spreads after 90 days of storage at 90° F. It seems probable that the final pH and hardness attained after storage actually reflect the maximum reasonable effect of the bacterial changes under the conditions of these experiments.

TABLE 5

Means of direct microscopic counts of bacilli and changes in mean penetrometer and pH values of spreads during storage for 90 days at 90°F. Each value is the mean of three lots

Modification [a]	Bacilli per gram [b]	Decrease in penetrometer	Decrease in pH
	(thousands)	*(0.1 mm.)*	*(pH)*
Base [c]	0.21	20	0.09
	Moisture adjusted		
Base − 3%	0	10	0.12
Base + 3%	0	16	0.07
Base + 6%	0.15	+4 [d]	0.06
	Fat adjusted		
Base + 3%	1,900.0	10	0.06
Base + 6%	0.18	4	0.13
	NDM substituted for CSNF		
3% NDM	44,000.0	16	0.21
6% NDM	10,000.0	57	0.21
	Lactose substituted for CSNF		
3% Lactose	28,000.0	70	0.40
6% Lactose	49,000.0	102	0.59

[a] Percentages are based on weight of finished cheese spread.

[b] Bacilli per gram derived from logarithmic averages of cell counts.

[c] Penetrometer and pH values of the base spreads at two days of age averaged 64 and 5.45, respectively.

[d] Penetrometer values averaged four units higher after storage.

Plating of aliquots of each of the ingredients used in making the spreads on dextrose tryptone agar failed to reveal the presence of gram-positive spore-forming bacilli. It is probable that the Dariworld actually was the source of the gram-positive bacilli, even though the samples examined failed to disclose them. Other lots of Dariworld made from the same milk supply by identical procedures have contained these organisms.

DISCUSSION

The several components of pasteurized process cheese spread usually varied in its formulation appear to have specific effects on firmness of body. The firmness decreased with increases in moisture, but the identity of the dry matter in the formula itself apparently influenced the extent of the change. Fat, within the range used in these experiments, had little effect upon firmness. Solids-not-fat, however, had an important firming effect. The solids-not-fat of cheese origin seemed especially significant to the firmness of the spreads. This effect probably is comparable to the firmer body characteristics of natural cheese

made from milk which has been modified by removal of fat or by addition of milk solids-not-fat.

Since moisture and cheese solids-not-fat had antithetical effects upon firmness, the proportions of these components to each other provided a more logical understanding of their influence on the finished spread. The definite trend of relationship between penetrometer values and ratios of moisture to cheese solids-not-fat shows the importance of recognizing this ratio in controlling firmness, even with wide variations in formulation.

It is recognized that the experimental evidence of this study was based on the use of semisoft Dariworld cheese. However, data accumulating in this laboratory indicate that spreads made from Cheddar cheese show the same trends of the relationship between H_2O/CSNF and spread firmness as found in this study. Jakubowski and Bijok, working with Tilsit cheese, also developed similar relationships which further suggest that the relationships demonstrated with Dariworld are not limited to this type of cheese.

The survival of microorganisms during processing presents a serious problem in subsequent storage of spreads. Certain defects which have been observed during storage of process cheese, such as gas formation, discoloration, and putrefaction, have been attributed to these organisms which survive processing heat treatments (4-6, 9).

In this study the common association of the maximum increases in firmness, large numbers of the gram-positive bacilli, and marked decreases in pH suggested the bacteriological origin of the hardening defect in the body of cheese spreads. The hardening may have been caused directly by changes in pH, since decreasing pH by adding lactic acid also causes marked increases in firmness of spreads (12). The more marked development of the defect in the lactose-rich spreads indicates the desirability of limiting the content of lactose when spreads are to be subjected to holding periods of 2 to 3 mo. at 90° F.

It is possible for the cheese processor to control body firmness of spreads by regulation of the H_2O/CSNF ratio through variations in composition. Various levels of firmness can be attained by altering the moisture content or by reducing the amount of cheese solids-not-fat by substitution with NDM, lactose, or dried whey. When these substitutions are made it is necessary for the processor to use proper methods of storage and formulation to avoid firming of spreads. This is especially true if firming is associated with decreasing pH and large numbers of bacilli.

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FACTORS AFFECTING THE pH-SENSITIVITY OF THE HEAT STABILITY OF MILK FROM INDIVIDUAL COWS

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SUMMARY

The heat stability of milk passes through a maximum and a minimum as the pH is increased from 6.4 to 7.0. The position of the maximum was displaced slightly towards the acid side with addition of phosphate, and slightly towards the alkaline side with addition of calcium, but the maximum heat stability attainable was only slightly affected by small additions (up to 3 m*M*/liter) of these ions.

The occurrence of the maximum and minimum in the pH-heat stability reaction was dependent upon the presence of β-lactoglobulin. The maximum heat stability of calcium-caseinate-phosphate complex resuspended in ultrafiltrate from milk increased, and the minimum decreased, with increasing addition of β-lactoglobulin from 0.1 to 0.6 mg. N/ml. However, it could not be concluded that β-lactoglobulin was the only factor influencing the pH-heat stability response in normal milks.

It is suggested that the denatured β-lactoglobulin-casein (or K-casein) complex is the pH-sensitive component.

In a previous paper (6), it was reported that the heat stability of milk from individual cows varied sharply with small adjustments in pH in the range 6.5 to 6.9. The extent to which the heat stability decreased from the maximum to the minimum with increasing pH (i.e., the pH-sensitivity) was greater for some cows than for others, and this suggested that the milks differed in their content of one or more factors that influenced the pH-sensitivity. An attempt was, therefore, made to enumerate and identify these factors, and the results are published herewith.

MATERIALS AND METHODS

Unless otherwise stated, the milk samples used in this study were taken from the same two cows, and the methods used were the same, as previously reported (6). The milk was skimmed by centrifugation, but was otherwise unprocessed except where a heat treatment is specifically reported in the text.

Three series of tests were made:

1. To determine the interrelation between pH and calcium or phosphate additions, series of 49 aliquots of a single milk were prepared covering all combinations of three levels of addition of hydrochloric acid (1.5, 3.0, and 4.5 m*M*/liter) and three levels of sodium hydroxide (1.0, 2.0, and 3.0 m*M*/liter), with three levels of calcium chloride (0.75, 1.5, and 2.25 m*M*/liter) and three levels of sodium phosphate (1.0, 2.0, and 3.0 m*M*/liter of mixed mono- and di-phosphate, pH 6.7). All samples were equilibrated overnight before determining the pH and heat stability.

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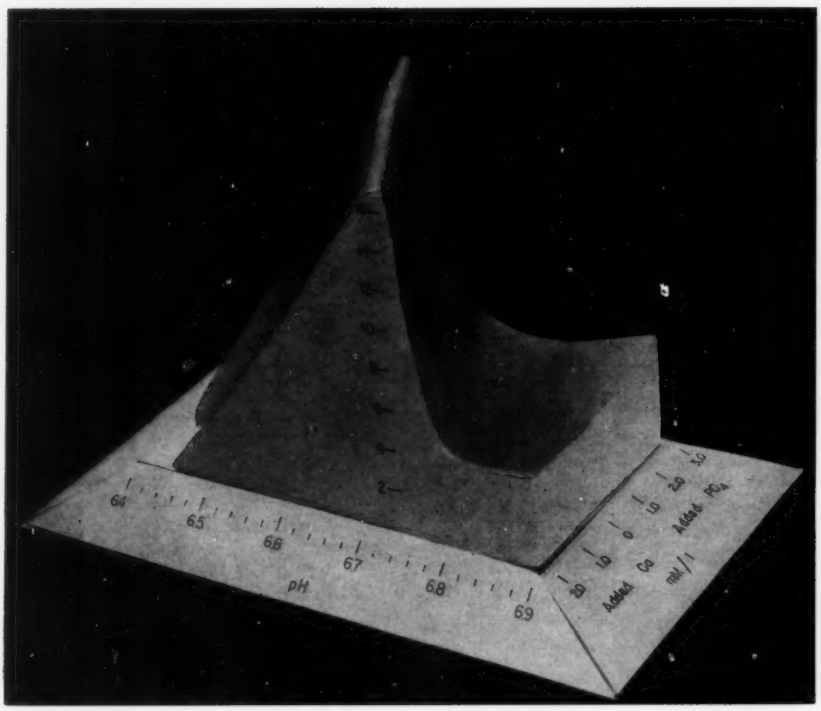

FIG. 1. The effect of additions of calcium and phosphate, and of pH adjustment on the heat stability (vertical scale, minutes) of milk from Cow 1.

2. To study the effect of interchanging caseins and sera, milk from each of the two cows was fractionated by centrifugation (1 hr. at *ca.* 80,000 × g) and the casein from each resuspended in serum from the other. This type of fractionation is, of course, incomplete because some serum is retained by the sedimented casein, so additional samples were prepared with caseins that had been washed once by suspending in serum from the other cow's milk, and recentrifuging. Control samples were prepared by sedimenting casein and resuspending it in its own serum. Aliquots of all these prepared milks were tested for heat stability over a range of pH values.

3. To study the effect of serum proteins, they were either denatured in situ by heating the milk to 95° C. for 10 min. (5), or milks essentially free of serum proteins were prepared by resuspending centrifuged casein in milk ultrafiltrate. Crude fractions of serum proteins were prepared by the method of Aschaffenberg and Drewry (2) (casein was first removed by centrifugation) and tested for their ability to impose a pH-sensitivity on these casein suspensions. Each of the fractions was dialyzed against water to remove most of the salt used in the preparation, then dialyzed against bulk milk, so that addition of the prepara-

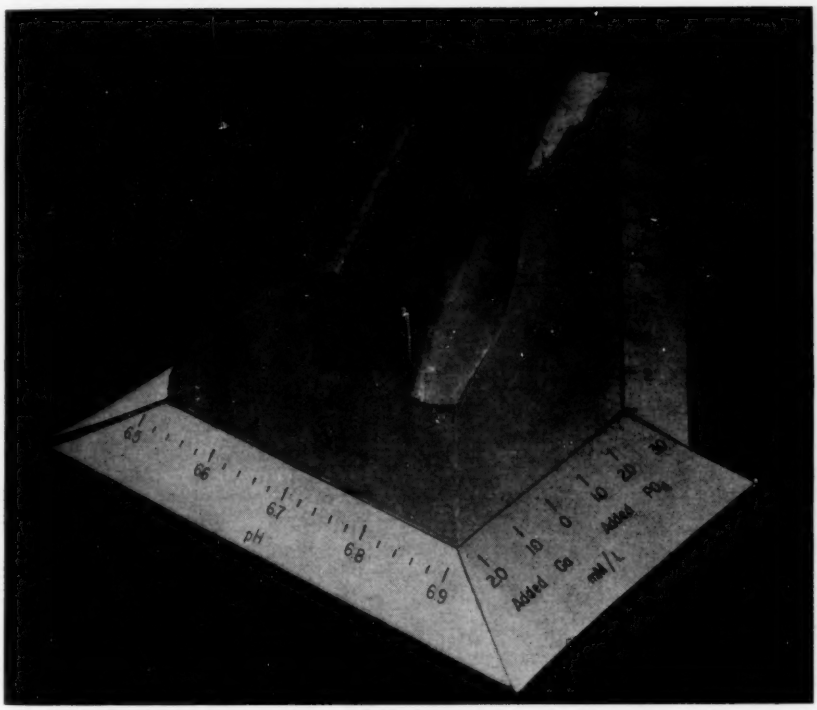

FIG. 2. The effect of additions of calcium and phosphate and of pH adjustment on the heat stability (vertical scale, minutes) of milk from Cow 2.

tions to ultrafiltrate had little effect on the inorganic composition. For subsequent tests, crystalline β-lactoglobulin was prepared (2) from the milk of both cows, and from bulk milk.

RESULTS

Interrelation between pH and calcium or phosphate additions. Results of the first series of tests (Figures 1 and 2) show that the effect of pH on the heat stability was not greatly changed, within the ranges studied, by addition of calcium or of phosphate, but that the pH of maximum heat stability [2] shifted towards the alkaline side as phosphate addition was decreased or calcium addition increased. In Figure 1, the minimum is broad and, within the range studied, no effect of added phosphate on the pH at which the second (more alkaline) increase in heat stability began is detectable; in Figure 2 it is apparent that this pH also decreased with increasing added phosphate. The maximum heat stability decreased slightly (½ to 1 min.) with increasing added calcium, and

[2] The term maximum heat stability is used to indicate the maximum attainable in the pH 6.5 to 6.8 range, although higher stabilities occur at pH levels above seven (6).

this effect became marked at still higher levels of added calcium (data not shown). With the samples used for the tests reported in Figures 1 and 2, there was an increase of ½ to 1 min. in the maximum heat stability with increasing addition of phosphate, but this effect was reversed at still higher levels of added phosphate. Both the pH of maximum heat stability, and the levels at which added calcium and phosphate began to decrease the maximum, varied between milk samples from the same cow, although the general shapes of Figures 1 and 2 were quite reproducible.

Effect of interchanging caseins and sera. Interchange of caseins and sera from the two cows showed (Figure 3) that the main factor(s) controlling heat stability were present in the serum and not in the casein. Suspending casein from Cow 1 in serum from Cow 2 gave a milk with a pH-sensitivity similar to that of normal milk from Cow 2, and vice versa. Centrifugation and redispersal

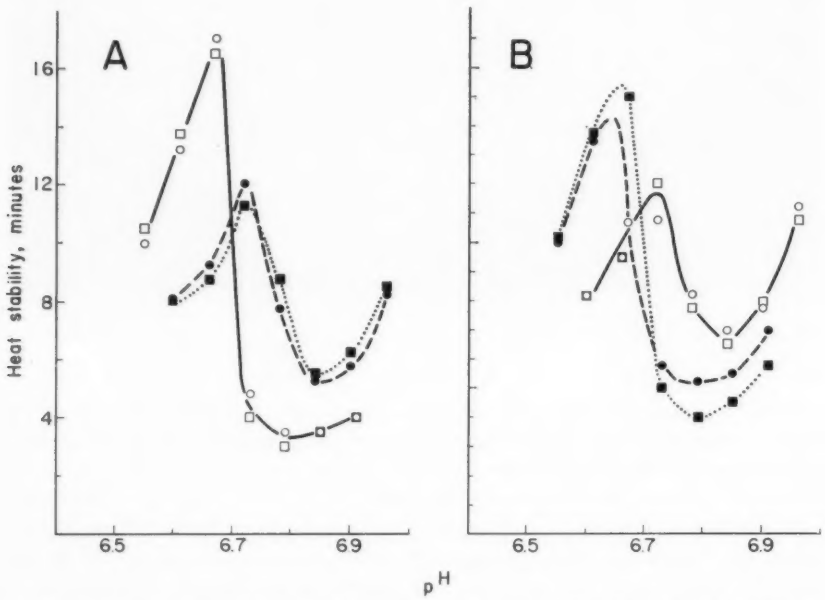

FIG. 3. Effect of suspending casein from Cow 1 in serum from Cow 2, and vice versa, on the relation between pH and heat stability.

Part A:

○—casein from Cow 1 in serum from Cow 1, single centrifugation.

□—as for ○, but centrifuged and resuspended twice.

●—casein from Cow 1 in serum from Cow 2, single centrifugation.

■—as for ●, but casein washed by centrifugation, resuspension, centrifugation, and resuspension in fresh serum.

Part B:

As for Part A, but with casein from Cow 2 and serum from Cow 2—○, □; or serum from Cow 1—●, ■.

in the same serum had little effect on the pH-sensitivity of milk from either cow (control samples, Figure 3).

In tests of this type it was difficult to be sure that losses of casein or serum during preparation of the samples did not cause variations in the concentration of casein. Tests were, therefore, made in which the casein concentration was deliberately varied from 70 to 130% of normal. The results showed that high concentrations of casein tended to lower, and low concentrations to raise, the maximum heat stability and the pH-sensitivity. However, with casein concentrations from 90 to 105% of normal, the effect on pH-sensitivity did not exceed the errors of interpolation of the pH versus heat stability curves. Casein concentration was, therefore, not an important factor in determining the pH-sensitivity of the milks used in these studies.

Effect of whey proteins. Denaturation of whey proteins by heating had little effect on the pH-sensitivity of milk (Figure 4), but removal of whey proteins (by resuspending centrifuged casein in ultrafiltrate) completely removed the pH-sensitivity. This absence of pH-sensitivity was observed consistently, regardless of the source of the casein or of the ultrafiltrate, and was also observed when sedimented casein was suspended in an artificial ultrafiltrate prepared from inorganic salts. The position of the curve relative to the pH scale varied between tests to about the same extent as the pH of maximum stability varied with normal milks, i.e., up to 0.2 pH unit. Further tests showed that heated milk responded to additions of calcium or phosphate in the same manner as raw milks. Casein in ultrafiltrate was not significantly affected by heating (Figure 4).

When the casein and serum of heated milk were separated by centrifugation and tested separately, pH-sensitivity factor(s) was sometimes found with the casein and sometimes with the serum (Figure 5). With most samples studied, the pH-sensitivity factor tended to sediment with the casein, but with samples from Cow 7 at least a portion of the pH-sensitivity was always found in the serum (Table 1). Considerable variation was also observed in the distribution of the acid-coagulable (denatured) serum protein of heated milks, and there was a slight relation between the distribution of denatured whey protein and of the pH-sensitivity factor (Table 1).

Of the three serum protein fractions tested (globulins, albumins, and β-lactoglobulin), only β-lactoglobulin reintroduced a pH-sensitivity into the suspensions of casein in ultrafiltrate. The effect of added crystalline β-lactoglobulin on the pH-sensitivity depended upon the amount added (Figure 6), especially in the range between 0.1 and 0.6 mg. β-lactoglobulin nitrogen per milliliter. Addition of less than 0.1 mg. N/ml did not significantly affect the heat stability of the suspension, but addition of larger amounts appeared to increase stability in the pH 6.5 to 6.7 range and to decrease it at slightly more alkaline levels. Addition of about 0.4 mg. β-lactoglobulin nitrogen/ml gave a milk with a pH-heat stability response similar to that for milk from Cow 1; addition of about 0.25 mg. N/ml gave a milk with a pH-response similar to that for milk from Cow 2.

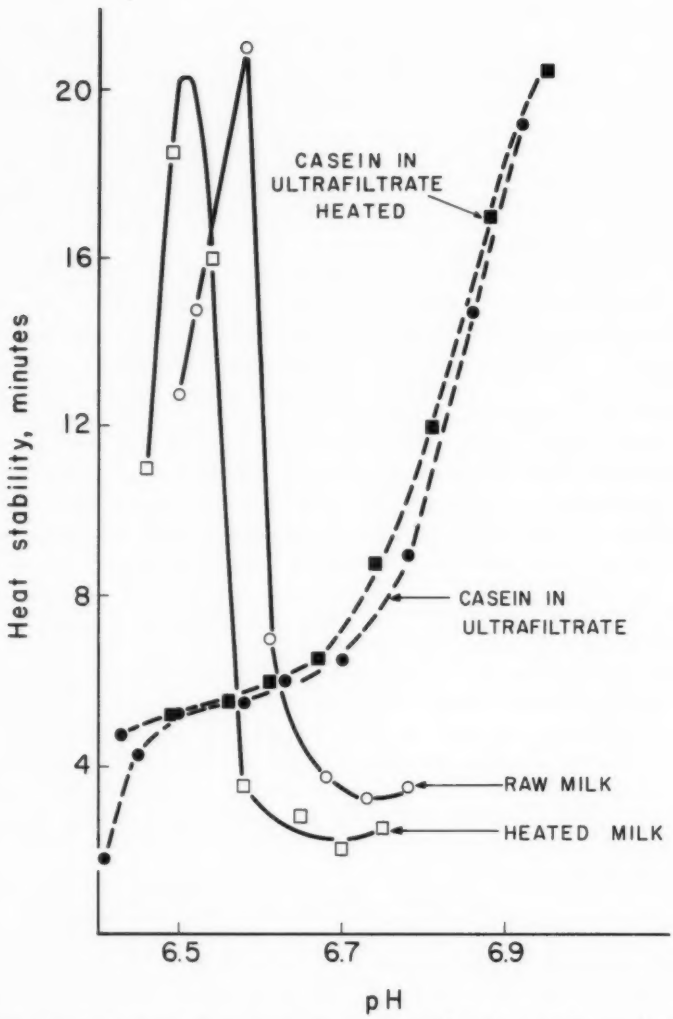

FIG. 4. Effect of a preliminary heat treatment at 95° C. for 10 min. on the heat stability of milk and of casein resuspended in ultrafiltrate.

TABLE 1

Distribution of pH-sensitivity factor and of denatured serum protein in heated, centrifuged milks

Cow No.	pH-sensitivity *(min.)*			Denatured serum protein [b]	
	A [a]	B [a]	C [a]	In serum	In casein
				(mg. N/ml)	*(mg. N/ml)*
4	15	0	7	0.05	0.49
5	12	0	5	0.18	0.36
5	16	0	6	0.00	0.65
6	7.5	0.5	2	0.12	0.40
1	8.3	2	1	0.10	0.35
7	14	6	2	0.12	0.69
7	15	12.5	0.5	0.59	0.36
7	14.7	11	0	0.32	0.63

[a] A—Control skimmilk.
B—Casein from raw milk resuspended in serum from heated milk.
C—Casein from heated milk resuspended in ultrafiltrate from raw milk.

[b] Denatured serum protein sedimented with casein was estimated from N content of centrifugal serum from raw milk minus N content of centrifugal serum from heated milk (4). Total denatured serum protein nitrogen was estimated from the increase in casein nitrogen precipitated at pH 4.7.

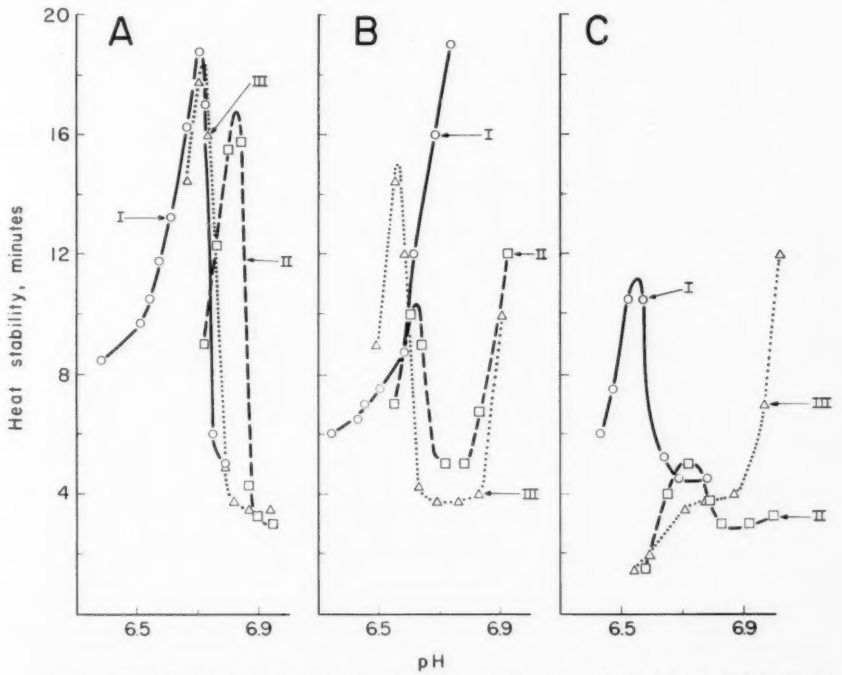

FIG. 5. Distribution of pH-sensitivity between casein and serum of heated (95° C., 10 min.) milks. Milk No. I from Cow 4; II and III from Cow 7.

A—Skimmilk control.
B—Casein from raw milk resuspended in serum from heated milk.
C—Casein from heated milk resuspended in ultrafiltrate.

Attempts to identify the type of β-lactoglobulin present (1) were not entirely satisfactory, but indicated that both Cow 1 and Cow 2 produced Type AB.

DISCUSSION

The results obtained in these studies show that the change in heat stability of milk with small alterations in pH was dependent upon the presence of β-lactoglobulin, and that the range of pH-heat stability response observed in normal milks (6) can be simulated in suspensions of calcium-caseinate-phosphate complex in milk ultrafiltrate by adding varying amounts of β-lactoglobulin. However, the difference in pH-heat stability response of milk from two cows previously studied was not related to variations in the total β-lactoglobulin present (6), and no difference was found in the type of β-lactoglobulin produced by these two cows. It must, therefore, be concluded that β-lactoglobulin is not the only factor influencing the pH-heat stability response.

The mechanism whereby β-lactoglobulin affects the pH-heat stability relation in casein resuspensions can not be fully explained at present. Presumably, β-lactoglobulin combines with casein, or a fraction (κ-casein) of casein [*cf.*

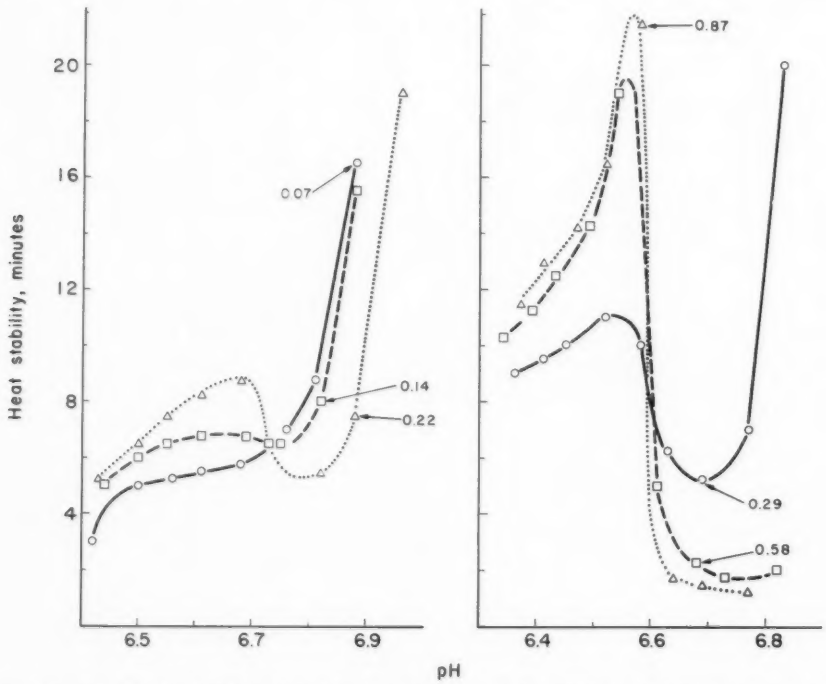

FIG. 6. Effect of β-lactoglobulin concentration on the pH-heat stability response of casein resuspended in ultrafiltrate. Figures beside curves indicate β-lactoglobulin additions calculated as mg. N/ml resuspension. Different milk samples from Cow 1 were used for the two sets of curves.

review by Tilley (8)] during the early stages of heating, and the protective action of this complex or its own rate of coagulation is highly dependent upon pH. However, the rapid decrease in heat stability when milk is adjusted to the alkaline side of the pH of maximum heat stability, and the subsequent increase, can not be readily explained by changes in the net charge on the denatured β-lactoglobulin-casein complex.

A preliminary heating of the milk can form the β-lactoglobulin-casein complex without altering its subsequent effect on the heat stability of the milk. With many of the milks studied, most of the complex thus formed sedimented with the casein, so that a pH-sensitivity was apparent in resuspensions of casein from heated milk in ultrafiltrate. However, with other samples most of the pH-sensitivity factor remained in the serum of heated, centrifuged milk, suggesting that in these samples the β-lactoglobulin had not formed a stable complex with casein. The distribution of denatured serum protein after centrifuging can not be expected to correlate closely with the distribution of pH-sensitivity, because of the difficulties involved in the determination of denatured serum protein (3, 7) and the fact that no distinction was made between the various denatured proteins.

Variations in the extent to which the pH-sensitivity factor and denatured whey protein sedimented with casein in these tests, and the temperature dependence observed by Sullivan *et al.* (7), suggest that dissociation of the β-lactoglobulin-casein complex may be more extensive and variable than indicated by electrophoretic analyses [*cf.* review (8)].

ACKNOWLEDGMENT

The author thanks the staff at the Dairy Barn, Central Experimental Farm, Department of Agriculture, Ottawa, Canada, for providing the milk samples used in these studies.

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FRACTIONATION OF THE LIPOPROTEINS OF THE FAT GLOBULE MEMBRANE FROM CREAM[1]

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SUMMARY

The membrane material obtained by freezing washed cream has been fractionated from suspensions in 2% sodium desoxycholate into five sedimentable fractions representing 75% of the total material and one soluble fraction (25%). These fractions were clearly differentiated by their appearance and sedimentation behavior. Fractions A and D contained most of the xanthine oxidase activity and were about 32% lipid. Fraction B contained most of the alkaline phosphatase activity and was about 65% lipid. The other fractions were over 70% lipid. In Fractions A, B, and D, the lipid was 15% phospholipid; in Fractions C and E, it was 5-6% phospholipid; and in the supernatant, it was 50% phospholipid.

The literature on the membrane of the bovine fat globule before 1955 has been reviewed comprehensively (11). Extensive fractionation and characterization studies have been carried out on the proteins of membrane material obtained after fat extraction [e.g., Herald and Brunner (7, 9)]. However, only limited work has been done on the lipoproteins as such. Enzymic and chemical analyses have been performed on milk microsomes, a small fraction of membrane material that sedimented in 15 min. at 50,000 × g but not at 10,000 × g (3, 4). Also, material obtained by churning unwashed cream, in which it is, therefore, difficult to determine the origin of the various fractions, has been fractionated with ammonium sulfate, and membrane material from washed cream was divided arbitrarily into two fractions by centrifugation (14). These fractions were analyzed only for alkaline phosphatase activity. The present paper reports the fractionation of the lipoproteins of most of the membrane material obtained from washed cream by freezing (8), and characterizes the fractions by enzyme content and chemical analyses.

MATERIALS AND METHODS

Fresh bulk milk, kept at a temperature higher than 30° C., was separated within 3 hr. after milking. The cream was washed and membrane material prepared essentially as described previously (8). The cream, approximately 50% fat, was frozen and stored at −10° C. in test tubes (1.4 cm. i.d.) for about 20 hr.; stored at −40° C. if not required immediately, never longer than 4 wk. After thawing the cream and diluting it fourfold with 0.01% gelatin solution, the membrane material was sedimented at 78,410 × g for 1 hr.

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Xanthine oxidase and alkaline phosphatase activities were measured as previously described (8). Nitrogen was estimated by micro-Kjeldahl (2) and total phosphorus by King's method (10). Lipids were extracted by the method of Bligh and Dyer (5); lipid phosphorus was estimated by a modified method (6) based on the hydrolysis technique of Allen (1) and on the color development of Polley (13), in which the perchloric acid was neutralized and the molybdate–trichloroacetic acid reagent was concentrated to enable the use of a larger volume of test solution.

Centrifugal forces are reported as average values from the Spinco Ultracentrifuge Technical Manual. Forces below 80,000 × g were obtained with a No. 30 head, higher ones with a No. 40, in a Spinco Model L centrifuge.

RESULTS

Fractionation procedure. Centrifugation of thawed cream yielded three layers [cf. (8)]: a small opaque pellet of the membrane material; an aqueous layer that contained only traces of enzyme activity or nitrogen; and an upper fatty layer. Some fluffy material was observed at the lower edge of the fatty layer; this material was easily resuspended in water and then sedimented at 78,410 × g for 1 hr. This sediment had xanthine oxidase and alkaline phosphatase activities, nitrogen and phosphorus, in proportions similar to those in the opaque pellet, but was not studied further.

During development of the fractionation procedure, preliminary tests indicated that the conditions used to redisperse the opaque pellet material (homogenization, ultrasonic treatment; suspension in sodium chloride, potassium chloride, or sucrose solutions, or in isobutanol) did not significantly affect subsequent fractionation by centrifugation. Fractionation was attempted by differential centrifugation of resuspensions made in the solvents mentioned above, and by means of an hydroxy apatite column, but the only method that gave satisfactory results was differential centrifugation of a suspension in at least 1%, and preferably 2%, sodium desoxycholate (DOC).

The procedure finally adopted (Figure 1) was carried out at 4% C., within three days from the thawing of the cream, as follows: Pellet material was obtained from about 1-1.5 liters of washed cream. The material from each 20 ml. of cream was homogenized in 1 ml. of 2% DOC, yielding a suspension containing about 5% total solids, and the suspension adjusted to pH 7.8. This suspension, centrifuged at 54,450 × g for 1 hr., yielded brownish pellets with a halo of slightly colored material. The lighter material was gently resuspended in the supernatant, and the mixture recentrifuged under the same conditions until no more brownish pellet sedimented (five times). All of the brownish pellets were suspended in fresh 2% DOC, combined and centrifuged (Fraction A).

Similar centrifuging-washing-recentrifuging procedures at succesively higher centrifugal forces yielded further fractions. Fraction B, the light-colored material described above, was sedimented from the combined supernatants from Fraction A at 78,410 × g for 1 hr. Fraction C, a white material, was obtained

by centrifuging the supernatant from B at 105,000 × g for 1 hr. The supernatant from Fraction C was finally centrifuged at 105,000 × g for 6 hr. to yield three fractions: D—a transparent, brown, firmly packed pellet; E—a loosely packed, creamy white sediment; and S—the supernatant.

All sedimented fractions were finally resuspended in a suitable volume of water for analysis.

Analysis of fractions. Five fractionations were carried out by the above procedure, and the average analytical results and standard deviations of the means are reported (Table 1). The standard deviations include, to a large extent, the variability introduced by the subjective steps involved in the fractionation procedure.

Fraction D was obtained only in the presence of DOC. In preliminary fractionations without DOC, all xanthine oxidase activity was found in the heaviest fraction, which must be the source of Fractions A and D. However, this separation was difficult to duplicate quantitatively, as indicated by the relatively large standard deviation for the xanthine oxidase activity in Fractions A and D ($\pm$ 8.7), in contrast with the value for the sum of A + D (85.1 $\pm$ 3.9%). Fractions A and D had the same lipid content (32%) and per cent phospholipid in the lipid fraction (15%). However, the specific activity of xanthine oxidase was much higher in D than in A. Fraction B, the major fraction, contained most of the alkaline phosphatase activity and had a high lipid content and a high phospholipid content. The two smallest fractions, C and E, were similar in appearance and composition and had the highest lipid content and lowest phospholipid content. The supernatant contained 25% of the membrane material, 29% of the lipid, and 50% of the phospholipid.

The neutral lipids of the fractions contained predominantly triglycerides, with free fatty acids, cholesterol, and monoglycerides in smaller amounts. The free and total fatty acids of the fractions obtained in one experiment were determined by gas-liquid chromatography on a Pye-Argon apparatus. The values are reported as mole per cent. The free fatty acid content of the fractions varied from 1 to 5% of the total lipids. The total fatty acids of the membrane material were lauric (2%), myristic (11%), palmitic (40%), oleic (14%), stearic (24%), with smaller amounts (approximately 1%) of volatile acids, C_{13} acids, palmitoleic and C_{15} acids. Fractions C and D contained 24 and 40% lauric acid, respectively. The final supernatant contained lauric (3%), myristic (3.5%), palmitic (12%), oleic (62%), and stearic (12%).

The phospholipid composition of the fractions of one fractionation was estimated by chromatography on silicic acid–impregnated paper and total phosphate estimation in spots identified by staining with Rhodamine G (12). In all fractions except D, 60-80% of the phospholipids was lecithin, with minor amounts of lysolecithin, sphingomyelin, lysocephalin, and phosphatidyl ethanolamine and serine. In Fraction D, 40% of the total phospholipid was lecithin, 30% phosphatidyl ethanolamine, and 20% phosphatidyl serine. The supernatant fraction contained slightly higher levels (15% total) of lysolecithin, lysocephalin, and sphingomyelin than did the other fractions.

No further characterization of the lipoprotein fractions by electrophoresis and ultracentrifugation was possible, because no suitable solvent was found. Most traditional lipoprotein solvents (i.e., sodium chloride, potassium chloride, isobutanol, sodium desoxycholate, doccanol, formic acid, urea) were tried without success.

DISCUSSION

The lipid composition and, particularly, the very low volatile fatty acids content of the membrane material studied indicate that it was not contaminated by milk fat.

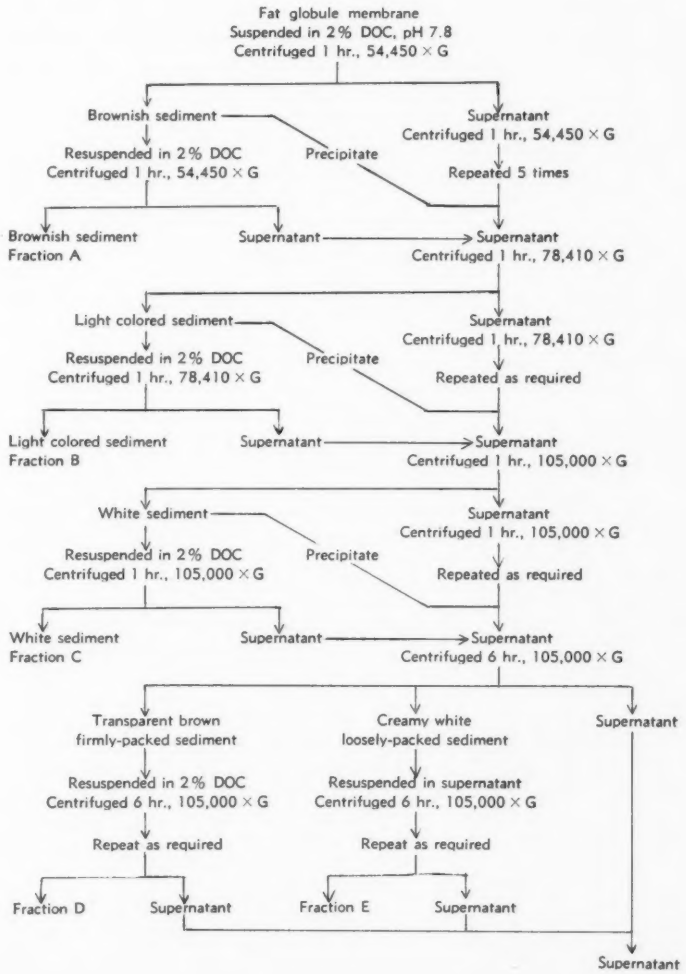

TABLE 1

Enzyme activity and composition of fractions from membrane material

Fractions	Amount of fraction,[a] % of original [d]	Xanthine oxidase activity [a]		Alkaline phosphatase activity [b]		Lipid [c] % composition [d]	Phospholipid [e] in lipid, % [g]
		% of original	Specific [e]	% of original	Specific [f]		
Original	100	100	135 ± 11	100	87 ± 30	60.2 ± 1.8	29.1 ± 5.6
A	24.9 ± 1.6	56.0 ± 8.7 [h]	173 ± 24	12.7 ± 2.1	26 ± 9	32.5 ± 2.5	15.0 ± 2.7
B	35.7 ± 3.7	10.4 ± 2.3	86 ± 15	65.1 ± 4.9	343 ± 122	64.8 ± 16.4	14.7 ± 2.3
C	1.4 ± 0.3	1.1 ± 0.5	84 ± 26	1.1 ± 0.2	108 ± 34	76.1 ± 3.8	5.7 ± 1.3
D	4.5 ± 0.7	29.1 ± 8.7 [h]	433 ± 83	3.3 ± 0.4	39 ± 3	32.4 ± 9.9	13.9 ± 6.4
E	3.5 ± 1.0	4.8 ± 2.2	155 ± 41	2.3 ± 0.8	84 ± 30	69.1 ± 0.5	5.1 ± 0.7
Supernatant	24.6 ± 4.6	3.4 ± 1.4	24 ± 8	3.2 ± 1.3	11 ± 2	70.2 ± 9.8	51.0 ± 20.0
Recovery	94.6	104.8		87.7			

[a] Averages ± standard deviation of the mean for five fractionations.
[b] Averages ± standard deviation of the mean for three fractionations.
[c] Averages ± standard deviation of the mean for two fractionations.
[d] Calculated from N × 6.25 and lipid weight, assuming that no other substance would significantly contribute.
[e] Micromoles of methylene blue reduced/hr/mg of nitrogen.
[f] Micromoles of phosphorus released/hr/mg of nitrogen.
[g] Assuming P × 25 = phospholipids.
[h] Per cent xanthine oxidase activity A + D = 85.1 ± 3.9.

Although obviously still impure, the fractions obtained in these tests were clearly differentiated by their appearance, their sedimentation behavior and their enzyme, total lipid, and phospholipid content. Sodium desoxycholate was necessary to separate the components. But even when suspended in 2% desoxycholate solution, about 75% of the membrane material was still sedimentable. Seventy per cent of the unsedimentable fraction was a lipid mixture containing 51% phospholipid.

The xanthine oxidase and alkaline phosphatase activities were closely associated with specific fractions, but neither was obtained free from the other. The lipid fractionation studies revealed a markedly different composition in Fraction D, which had a high lauric acid and phosphatidyl ethanolamine and serine content, and in the supernatant fraction (high phospholipid content), but again the fractionation was very incomplete. No relation between the composition of a fraction and its affinity for either of the enzymes is apparent.

ACKNOWLEDGMENTS

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SOME FACTORS INFLUENCING THE ACTIVITY OF THE A-, B-, AND C-ESTERASES OF BOVINE MILK[1]

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SUMMARY

Methods of independently assaying milk for A-, B-, and C-esterase activity, using Warburg manometric techniques, are described in detail. Activities are expressed as A_{30}, i.e., the μl. of CO_2 evolved by 1 ml. milk during 30 min. of incubation under prescribed conditions of temperature, pH, substrate, and inhibitor.

Using these assay methods, average A_{30} values for the three esterases were found to be: A, 60; B, 234; and C, 20. First-milking colostrum differed greatly in the activity of these esterases. Mean A_{30} values for 11 samples of first-milking colostrum were 424, 20, and 320 for the A-, B-, and C-esterases, respectively. A-esterase activity dropped to an average level in about a month following parturition, B-esterase activity grew to average in about 4 mo., and C-esterase activity dropped to average in about three days.

Milk from mastitic quarters was found to be significantly higher in A-esterase activity than that from normal quarters. It is suggested that this difference might serve as the basis of a screening test for mastitis.

Activities of the three esterases were not significantly different between first- and last-drawn milk and, with the exception of mastitic quarters, were not consistently different between quarters.

The B-esterase activity of normal milk appears to be an individual characteristic, and may vary between individuals by a factor of four or more.

Aldridge (1), in 1953, reported the presence of two distinct types of esterases in the sera of many species, in addition to cholinesterase. These two types were differentiated on the basis of their ability to hydrolyze *p*-nitrophenyl acetate, propionate, and butyrate and their sensitivity to or tolerance of certain organophosphorus enzyme inhibitors. Both types had the ability to hydrolyze the *p*-nitrophenyl esters mentioned above, but the relative rate of hydrolysis of the esters varied between the two esterases. The A-type would tolerate 10^{-3} *M* concentrations of the inhibitors, whereas the B-type was completely inhibited by much lower concentrations (10^{-8} to 10^{-7} *M*). In 1958 and 1959, after a comprehensive survey of the plasma of vertebrates for A- and B-esterases and cholinesterases, Augustinsson (2, 4-6) proposed the following classification:

A-esterases (arylesterases): Typical aromatic esterases which hydrolyze phenyl acetate at a higher rate than phenyl butyrate. Aliphatic esters are normally not attacked. Resistant to both organo-phosphorus compounds and physostigmine (eserine).

B-esterases (aliphatic esterases, lipases): Hydrolyze both aliphatic and aromatic esters but not choline esters. Sensitive to organo-phosphorus com-

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pounds, but resistant to 10^{-5} *M* physostigmine. Some B-esterases are sensitive to higher concentrations of the latter compound.

C-esterases (cholinesterases): Choline esters are split at a higher rate than both aliphatic and aromatic esters, the latter usually being hydrolyzed at a lower rate than aliphatic esters, or not at all. Sensitive to organo-phosphorus compounds and physostigmine, the latter giving 100% inhibition in 10^{-5} *M* or lower concentration.

About this same time, Augustinsson (3) reported the presence of a cholinesterase (C-esterase) in colostrum of the dog and swine, and Montoure *et al.* (14) showed such an enzyme to be present in colostrum of the cow. Augustinsson and Olsson (7, 8) also showed that swine milk contained both a B- and a C-type esterase. Finally, in late 1959, Forster *et al.* (10) reported that bovine milk contained esterases of all three types and suggested an assay procedure which might be used to measure the activity of each of these esterases independently of the other two.

This report describes, in some detail, the assay procedure mentioned above. It also identifies some of the factors influencing the activity of each of the three esterases present in bovine milk.

EXPERIMENTAL METHODS

Collection and preservation of milk samples. Milk samples were collected, usually at the evening milking, in amber glass bottles, immediately placed in ice water, and brought to the laboratory where they were gassed with nitrogen for 20 min. (11). They were then stored in an atmosphere of nitrogen in the refrigerator (0 to 3° C.) until assayed. Unless otherwise indicated, samples were representative of the entire milking. Assays were usually made the following day but, in some instances, two days elapsed before assays were completed.

Preparation of substrates and inhibitors. 1.0 M emulsions of phenyl acetate, phenyl propionate, and tri-n-butyrin. These emulsions were prepared by weighing 6.8 g. of phenyl acetate, 7.5 g. of phenyl propionate, or 15.1 g. of tri-*n*-butyrin, depending upon which substrate was being prepared, and 1.5 g. of Triton X-155 into a 50-ml. volumetric flask and mixing. Two milliliters of gum acacia solution (2.5 g. per 100 ml. solution) were added and mixed and the volume made to the mark with distilled water. After thorough mixing, the contents of the flask were transferred to a semimicro Waring Blendor container and homogenized for 5 min. at the top speed of the Waring machine.

3% Triton X-155. 3.0 g. of Triton X-155 were weighed into a 100-ml. volumetric flask and 4.0 ml. of gum acacia solution (2.5 g. per 100 ml. solution) added and mixed. The volume was made to the mark with distilled water and mixed thoroughly.

4×10^{-6} M tetraethyl pyrophosphate (TEPP). About 12 to 15 mg. of TEPP were transferred (with suitable precautions) to a weighed 10-ml. volumetric flask and weighed exactly. The volume was made to the mark with distilled water and the exact molarity calculated. A 4×10^{-6} *M* solution of TEPP was prepared from this (approximately 4×10^{-3} *M*) solution by suitable dilutions.

4×10^{-2} M Parathion emulsion. About 300 mg. of Parathion were transferred (with suitable precautions) to a weighed 25-ml. volumetric flask and weighed exactly. Two milliliters of gum acacia solution (2.5 g. per 100 ml. solution) were added and mixed and the volume made to the mark with 1% (W/V) Triton X-100 solution. After thorough mixing, the contents of the flask were transferred to a 250-ml. glass-stoppered bottle. A calculated volume of 1% Triton X-100 was then added to dilute this emulsion to exactly 4×10^{-2} *M* in Parathion. This emulsion separated readily; hence, it was always shaken vigorously just before use.

The substrate emulsions described above were prepared as needed and stored in the laboratory refrigerator, except when being used to set up an assay. Both phenyl acetate and phenyl propionate were synthesized in this laboratory and purified by careful distillation. Tri-*n*-butyrin was obtained from Eastman and was used as received. TEPP was obtained from Bios Laboratories and was considered pure. Solutions of this inhibitor were prepared fresh each day and were stored in the refrigerator when not actually being used to set up a run. Monsanto kindly donated the Parathion. It, too, was considered to be pure. Fresh emulsions of this inhibitor were prepared at least every second day and were stored in the refrigerator when not in use. Milk and inhibitor were in contact for at least 30 min. prior to start of the enzyme activity measurement. During preparation and pipetting of these inhibitor solutions, and in washing up, precautions were taken to guard against ingestion, inhalation, or contact with the skin (propripette, rubber gloves, hood).

Measurement of esterase activity. Activity of each of the three esterases was measured at 37° C. and pH 8.0 during a 30-min. incubation, using Warburg manometric techniques (15). Fluid volume was 4.0 ml. After mounting on the water bath, the manometers with attached reaction vessels were gassed (5%, CO_2, 95% N_2) for 10 min. and equilibrated for at least 10 min. before starting the reaction by tipping in the contents of the side arm. The essential set-up used to assay a single sample of milk for each of the three esterases is shown in Table 1. Actually, each flask set-up shown in this table was duplicated and the entire 12 flasks, adequately randomized, were handled as a single run on the Warburg.

It will be observed, from an examination of Table 1, that A-esterase activity of milk was measured in the presence of 10^{-2} *M* Parathion, with phenyl acetate as substrate. Use of the inhibitor was necessary because this substrate is also hydrolyzed by B- and C-esterases, both of which are completely inhibited by 10^{-2} *M* Parathion (10). Flask 2 (Table 1), containing heated milk, served as the blank to correct for nonenzymatic hydrolysis of phenyl acetate and a slight tipping error. No correction for milk fat hydrolysis was required, because the Parathion present effectively inhibited such hydrolysis (9).

Tributyrin served as substrate to measure B-esterase activity of milk. The reaction was carried out in the presence of 10^{-6} *M* TEPP. This completely inhibited C-esterase hydrolysis of tributyrin (10, 14). A-esterase has been shown not to hydrolyze this substrate (10). Use of raw milk in the blank (Flask 4,

TABLE 1

Set-up of Warburg flasks used to assay a single sample of milk for A, B, and C-esterase

	Esterase					
	A		B		C	
Substrate	PA[a]	PA	TB[b]	TB	PP[c]	PP
Flask No.	1	2	3	4	5	6
3% TX-155 *(ml.)*	....	....	....	0.4	....	
1.0 *M* Substrate *(ml.)*	0.4	0.4	0.4	....	0.4	0.4
0.2940 *M* $NaHCO_3$ *(ml.)*	0.1	0.1	0.1	0.1	0.1	0.1
Above in side arm below in main compartment						
0.1372 *M* $NaHCO_3$ *(ml.)*	1.5	1.5	1.5	1.5	1.5	1.5
4×10^{-6} *M* TEPP[e] *(ml.)*	....	....	1.0	1.0	....	1.0
4×10^{-2} *M* Parathion *(ml.)*	1.0	1.0	....	....	....	....
H_2O *(ml.)*	....	....	....	....	1.0	....
Milk treatment[d]	R	H	R	R	R	R
Milk *(ml.)*	1.0	1.0	1.0	1.0	1.0	1.0

[a] PA = phenyl acetate.
[b] TB = tri-n-butyrin.
[c] PP = phenyl propionate.
[d] R = raw, H = heated at 75° ± 5° C. for 10 min.
[e] 4×10^{-5} *M* Eserine can replace the TEPP.

containing no substrate, Table 1) provided a correction for slight milk fat hydrolysis and a slight tipping error. It was found that nonenzymatic hydrolysis of tributyrin was insignificant and could be ignored.

C-esterase activity was determined, using phenyl propionate as substrate. In this case, that part of the hydrolysis of phenyl propionate which was inhibited by 10^{-6} *M* TEPP was ascribed to C-esterase activity (Table 1, Flask 5 activity minus Flask 6 activity), since this concentration of TEPP does not inhibit either of the other two enzymes (10). The presence of raw milk and phenyl propionate in both Flasks 5 and 6 (Table 1) automatically compensated for enzymatic hydrolysis of milk fat, nonenzymatic hydrolysis of phenyl propionate, and any slight tipping error.

Calculation and expression of enzymatic activity. The average μl. CO_2 evolved from each pair of blanks was calculated. This value was subtracted from the μl. CO_2 evolved from each of the other two flasks in an assay; thus, duplicate values for net μl. CO_2 evolved were obtained. These values were assumed to represent the activity of a particular esterase in 1 ml. of a particular sample of milk during 30 min. of incubation under the specific assay conditions. Throughout this report such net μl. CO_2 evolved are referred to as A_{30} (activity in 30 min.). In a few instances, where the activity was abnormally high, A_{30} values were calculated from actual activities observed for only 10 or 20 min. of incubation. In a few other cases, when assaying very viscous samples of colostrum, A_{30} values were calculated from actual activities observed, using samples diluted with water.

RESULTS

Herd survey. The esterase activity levels of milk from 41 cows in the University herd were surveyed. The purpose of this experiment was to establish

an average activity level and range in activity for each of the three esterases. Eight Guernsey, 17 Jersey, and 16 Holstein cows comprised the 41 whose milk was assayed in the herd survey. Days in milk and weight of milk produced at the time of sampling were recorded and all samples were tested for fat by the Babcock method after assays were completed.

The data obtained in the herd survey are summarized in Table 2. It is evident from this table that average A_{30} values for the three esterases differed markedly. It is evident, also, that A_{30} values for A- and B-esterases varied over a wide range, from 20 to 198 for A-esterase and from 55 to 585 for B-esterase. An interesting and significant observation concerning the data in Table 2 is that the maximum A-esterase value of 198 and the minimum B-esterase value of 55 were obtained from a sample of milk collected four days following calving. The original data of this experiment were classified by breed, fat content of milk, weight of milk, and days in milk. A_{30} values for each of the three esterases were not appreciably influenced by any of these factors. However, four samples collected very early in the lactation period (four to 16 days), did show abnormally high A- and abnormally low B-esterase activities. Average A_{30} values for these four samples were 149 and 91 for the A- and B-esterases, respectively.

TABLE 2

Summary of A-, B-, and C-esterase activity values of milk from 41 cows

Esterase	A_{30} values Minimum	Maximum	Mean
A	20	198[a]	60
B	55[a]	585	234
C	10	34	20

[a] Four days postpartum milk.

Stage of lactation. Results described in the preceding paragraph indicated a strong possibility that activities of at least two of the three esterases might be markedly different from average during the immediate postpartum period. To study this possibility, colostrum samples were collected on the day of calving (as soon as possible after calving) and on the following three days. Milk samples were then collected at about weekly intervals for the remainder of the first month and at about monthly intervals for the remainder of the lactation period or until the cow was removed from the herd. The first-milking colostrum samples, i.e., those collected as soon as possible after calving, generally were not truly representative. Efforts were made to collect some colostrum from each teat, but this was not always possible and in some instances the entire sample was obtained from one quarter only. Later samples were obtained from the milker bucket and were representative of the colostrum or milk which had been obtained at that milking. All samples so collected were preserved and assayed according to previously described methods. The colostrum and milk of seven Holstein and four Jersey cows were studied in this manner.

A summary of the esterase activities of the 11 samples of first milking colostrum is in Table 3. Mean A_{30} values for the three esterases, shown by

TABLE 3

Summary of A-, B-, and C-esterase activity of 11 first-milking[a] colostrum samples

Esterase	A_{30} values Minimum	Maximum	Mean
A	175	1,141	424
B	4	35	20
C	25	853	320

[a] Collected 10 min. to 8 hr. postpartum.

these colostrum samples, are strikingly different from mean values for normal milk, shown in Table 2. So also are the ranges, as represented by differences between minimum and maximum values. It is evident that first-milking colostrum usually possesses very high A- and C-esterase activity and very low B-esterase activity.

The changes which take place in the activities of these esterases as the lactation period progresses are evident from the summarized data in Table 4. Unfortunately, samples were not always collected at the correct time to fall into the time categories shown in the first column of this table. This, together with the disposal of some cows before the study was completed, resulted in somewhat greater variability in the data than otherwise would have been the case. In spite of this added variability, however, the major changes are strikingly evident.

TABLE 4

Influence of stage of lactation on activity of A-, B-, and C-esterases

In milk	No. of observations	Mean A_{30} values for esterase A	B	C
(days)				
0	11	424	20	320
1	10	290	52	147
2	11	177	80	45
3	9	204	78	36
6–15	11	116	161	20
16–25	9	107	164	13
(months)				
1	11	91	166	17
2	11	67	161	15
3	11	59	205	18
4	11	60	247	17
5	9	57	288	18
6	11	73	238	17
7	9	122[a]	254	19
8	8	74	255	20
9	8	59	252	17
10	7	47	260	17

[a] One cow had severe mastitis.

Attention is directed to the high A-esterase activity at the seventh month. This was due to an A_{30} of 583 for one of the eight samples included in the mean of 122. The mean for the other seven samples was 64. This one sample was from a cow which had just suffered a serious mastitis attack.

Mastitis. The possibility that mastitis might have been responsible for the high A-esterase activity mentioned in the preceding paragraph was investigated in the experiment described below. Ten mastitic and normal, paired milk samples were collected from seven different cows having mastitis in only one quarter of the udder. About 50 ml. of milk were withdrawn, by hand, from the mastitic quarter, directly into a sample bottle. Immediately thereafter, a control sample of about 150 ml. (50 ml. from each of the other three quarters) was obtained in the same manner. These samples were obtained just after the cow had been prepared for milking and before the milking machine was attached. The presence of mastitis was judged by an experienced milker and was based on the presence of flakes on the strip cup, swelling of the quarter, or both. Cases involved ranged from very mild to very severe. All samples were preserved and assayed for esterases by the methods previously described.

TABLE 5
Influence of mastitis on activity of A-, B-, and C-esterases

Type of milk	No. of observations	Mean A_{30} values for esterase		
		A	B	C
Mastitic	10	652	175	25
Normal	10	66	237	24

Table 5 summarizes the data of this experiment. In every pair of samples in the original data, A-esterase activity of the mastitic sample substantially exceeded that of the control. Analysis of variance of the data indicated that this difference was significant at the 5% level. The A_{30} values for B-esterase activity shown in Table 5 are somewhat misleading. In four of the ten comparisons, the mastitic sample was slightly higher in B-esterase activity than the normal sample. In four of the remaining comparisons it was slightly lower. In two comparisons, involving samples from one cow, No. 2168, B-esterase activity of the mastitic samples was very low, comparable with first-milking colostrum ($A_{30} = 49$ and 17); the control samples had normal activity ($A_{30} = 235$ and 350). Such results definitely were not typical and the data of Table 5 should be considered with this in mind. Analysis of variance indicated that the difference between B-esterase activity of mastitic and normal milk was not significant at the 5% level. However, a significant difference certainly did exist in the case of the samples from Cow 2168, mentioned above.

First- and last-drawn milk. A comparison was made of the activities of the three esterases from first- and last-drawn, paired samples of milk from ten different cows. In collecting these samples, the cow was prepared for milking in the usual manner. About 15 ml. of milk were then drawn by hand from each quarter, into a sample bottle, to become the first drawn sample of the pair. The cow was then machine-milked, but milking was interrupted before all milk had been removed from the udder. A second sample, the last drawn, was then obtained in a manner similar to the first. The pair of samples thus collected was preserved and assayed as previously described.

TABLE 6

Activity of A-, B-, and C-esterases of first- and last-drawn, paired samples of milk from ten different cows

Milk sample	Mean A_{30} values for esterase		
	A	B	C
First	52	170	18
Last	46	169	18

Table 6 summarizes data collected in this experiment. It will be observed that the only appreciable difference was in the A-esterase activity. Analysis of variance indicated this difference was not statistically significant.

Different quarters. It was of interest to learn if variations of a significant magnitude, in the activity of any of the three esterases, might be encountered in milk samples from different quarters of the udder of an apparently normal healthy cow. Individual quarter samples of about 75 ml. were drawn by hand after the cow had been prepared for milking in the usual manner. These samples were preserved and assayed in the manner previously described. The order of assay of each group of four quarter samples was randomized to eliminate bias due to length of storage. Nine trials were made, involving individual quarter samples from four different cows.

The magnitude of the variations in A- and C-esterase activity between quarters, revealed by this experiment, could very well have been caused by random errors of the assay procedure, with the exception of one quarter of the udder of one cow. Milk drawn from this quarter on three separate occasions showed A-esterase activity three to four times greater than that of any of the other three quarters. Inquiry revealed that the teat of this quarter, the left rear, had been injured in a previous lactation.

Variations in B-esterase activity of milk from different quarters were often substantially greater than could be explained on the basis of errors of assay.

TABLE 7

B-esterase activity of milk from different quarters of the udder

Cow No.	A_{30} values			
	Front		Rear	
	Left	Right	Left	Right
3175	346	362	327	318
520	254	265	217	250
520	321	309	282	308
483	103	126	92	90
483	90	110	95	50
483	64	83	64	62
2162	691	760	575	571
2162	826	662	434	724
2162	776	885	570	663
Means	386	396	295	337
	391		316	

Sampling errors might have been responsible for a part of this variation, however. Average B-esterase A_{30} values for each quarter sample assayed are shown in Table 7. Substantial differences between quarters are evident, particularly in the samples from Cow 2162. In every instance, the front half samples averaged higher in B-esterase activity than did the corresponding rear half samples (Table 7). Analysis of variance indicates the difference was significant ($F = 9.15$, 5% point of $F = 5.32$).

Individuality of the cow. Previous sections of this report have shown little variation in A- and C-esterase activity of milk from individual cows unless abnormal (mastitic or early lactation) milk has been involved. However, considerable variation in B-esterase activity has been evident. The B-esterase activity of milk from 28 different cows, included in the herd survey, is shown in Table 8. Only those cows which had been milking more than 100 days are in

TABLE 8

B-esterase activity of milk from apparently normal cows, classified by breeds

Holstein		Jersey		Guernsey	
Cow No.	A_{30}	Cow No.	A_{30}	Cow No.	A_{30}
457	121	2162	501	3135	369
390	236	2199	273	3154	232
451	130	2212	194	3153	196
494	251	2220	585	3151	158
284	290	2139	127	3133	277
490	174	2238	288		
402	381	2167	152		
452	128	2197	277		
448	429	2241	178		
320	219	2198	312		
		2159	151		
		2233	256		
		2225	191		

this tabulation. Elimination of cows which had been milking for a shorter time removes most of the influence of early lactation. The activities have been classified by breed to illustrate that high and low activities occur within each breed.

Spontaneous rancidity. All of the milk samples collected in the herd survey, 41 from 41 different cows, and all of those collected in the stage of lactation study, 173 from 11 different cows, were examined for rancid flavor after 24 hr. of refrigerated storage. Not a single instance of rancidity was encountered in these 214 samples of milk.

DISCUSSION

The assay methods used in obtaining the data reported herein can not be considered as absolute measures of the amount of each esterase present. Though the report has been written as if only the activity of three specific esterases were being estimated, the authors are well aware of the fallacy of this viewpoint. Actually, several similar esterases may be hydrolyzing each of the substrates used in the assay procedures in the presence of the specific inhibitor conditions.

Further, these assay procedures are essentially arbitrary. Preparation of substrate emulsions and selection of incubation and pH conditions represent arbitrary decisions. If these conditions are varied, activities measured will vary also. Efforts were made, in setting up the assay procedures, to correct for certain errors. Spontaneous hydrolysis of the substrates and of milk fat and any slight tipping errors, other than purely random ones, have been taken care of by suitable blanks. One source of error has been neglected. This is the error resulting from the binding of the acids, resulting from substrate hydrolysis, by the buffers normally present in the milk. Acids so bound would not release CO_2 from the bicarbonate buffer used in the assay. This error would result in lowered A_{30} values. However, it would be reasonably constant from milk to milk. Gross changes in the buffering capacity of milk might be expected when assaying colostrum or mastitic milk. These changes would shift the magnitude of this error. The assay procedures also make no provision for the possible presence of natural inhibitors or activators in the samples. In spite of these weaknesses and errors, the methods provide for a reasonable degree of precision. The authors are convinced that many of the differences in activity revealed by the use of these methods are real, though of course, relative, rather than absolute differences.

Some evidence now at hand indicates that B-esterase of milk is a component of the milk lipase system. It has been established, for instance, that B-esterase hydrolyzes milk fat (9) in addition to tributyrin. Herrington and Guthrie (12), using acid degree increases on milk samples refrigerated 24 hr. as their measure of enzyme activity, reported that milk lipase activity was very low in milk secreted during the first week of lactation. The low B-esterase activity of colostrum, reported herein (Tables 3 and 4), supports and explains the findings of Herrington and Guthrie (12), if one assumes that B-esterase and milk lipase are essentially the same enzyme. The results of Mattick and Kay (13) seem to conflict with this view. They reported that the tributyrinase content of milk from individual cows was highest in the colostrum, fell to a minimum on about the tenth day, then later rose to about midway between this value and the colostrum value. Mattick and Kay used tributyrin as substrate, with no added inhibitor. Their observations can be explained on the basis of the combined activity of both the B- and C-esterases. C-esterase is usually very high in colostrum (Tables 3 and 4) and hydrolyzes tributyrin (10, 14) at an appreciable rate, whereas B-esterase is very low in colostrum (Tables 3 and 4). The high initial tributyrinase activity of colostrum on the first day, reported by Mattick and Kay, would be due almost entirely to C-esterase activity, since no C-esterase inhibitor was present. By the third or fourth day after calving, the contribution of C-esterase to tributyrinase activity would have dropped to a low value. That of the B-esterase would be on the increase (Table 4). At about this time one would expect to observe the lowest tributyrinase activity. Critical examination of the curves presented by Mattick and Kay reveals that the low point in activity was actually reached at this time rather than the tenth day.

During the experiment with mastitis, some samples of mastitic milk had

A-esterase activities as much as 50 times the average level. This suggests the possibility of using A-esterase activity as a factory screening test for mastitis. It should be possible to develop a simple colorimetric procedure, somewhat similar to the phosphatase test, to assay for A-esterase, since phenol is a hydrolytic product of the substrate, phenyl acetate.

The authors realize the limited scope and inadequacies of several of the experiments described in this report. Their objective, however, was to identify factors responsible for major variations in the activities of the three esterases. More comprehensive and better controlled experiments may demonstrate smaller, but statistically significant, differences where the experiments reported here have failed to do so.

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CHEMICAL CHANGES IN BACTERIA HEATED IN MILK AS RELATED TO LOSS OF STAINABILITY

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SUMMARY

The effect of heating bacteria in milk on direct microscopic clump counts was determined. The periodic acid–bisulfite-toluidine blue (PST) and the aniline oil–methylene blue (AOMB) stains were compared. The PST stain gave quantitative recovery in all cases, whereas the AOMB procedure did not stain gram-negative rods that had been heated. Gram-negative rods were poorly stained by the PST method, but could be counted. The proteose-peptone fraction of milk was primarily responsible for the loss of stainability. Mineral salts were less effective. Loss of stainability was associated with extraction from the bacterial cells of nitrogen and phosphorus-containing materials, identified as nucleic acids. The superior results obtained with the PST stain are attributed to the fact that polysaccharides as well as nucleic acids are stained. Strains of bacteria which contain little polysaccharide stain poorly by this procedure after nucleic acids are removed.

It has been frequently observed that bacteria tend to lose their stainability after they have been heated in milk. When direct microscopic clump counts (DMC) of bacteria are used to grade nonfat dry milk, poor staining sometimes makes accurate and reproduceable counting difficult, and some of the dead bacteria probably do not stain at all. There is, therefore, some doubt as to whether the DMC is a valid indicator of the sanitary quality of the raw milk from which the dry milk has been processed.

Recently, the author described a staining procedure (4) for bacteria in milk, based on treatment of the smear with periodic acid, bisulfite, and toluidine blue dye, which showed promise of overcoming some of the shortcomings of conventional methylene blue stains (7). Some minor modifications of the earlier procedure have been made which shorten and simplify it. The present paper gives data comparing the performance of the new procedure (PST) with that of the standard aniline oil–methylene blue (AOMB) stain, and also presents a study of chemical changes which take place when bacteria are heated in milk and which account for the poor stainability of bacteria in heated milk products.

METHODS AND MATERIALS

A. Reagents

1. Fixative—three parts absolute ethanol; one part glacial acetic acid prepared immediately before use.
2. Periodic acid—0.5 g. reagent grade in 100 ml. water.
3. Bisulfite solution—5 g. sodium or potassium metabisulfite in 100 ml. water.
4. pH 4 toluidine blue—0.1 g. of toluidine blue 0 dye in 100 ml. of

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pH 4 of MacIlvaine's buffer (prepared from 38.5 ml. of 0.2 *M* disodium phosphate solution mixed with 61.5 ml. of 0.1 *M* citric acid).

5. Schiff's Reagent—a sensitive Schiff's reagent can be prepared by avoiding a large excess of acid (1) and SO_2 (1, 9) in the solution. A satisfactory formula is, suspend 0.5 g. of basic fuchsin in 100 ml. distilled water and add 6 ml. of *N* HCl. The dye should completely dissolve. Then add 5 ml. of 15% sodium or potassium metabisulfite and shake at intervals until the solution is straw-colored, decolorize with charcoal, and filter. Store in a tightly closed bottle.
6. Sulfurous acid rinse solution—add 10 ml. *N* HCl and 10 ml. 15% sodium or potassium metabisulfite to 180 ml. water. Store in a tightly closed bottle.

B. Staining procedures

1. Periodic acid–bisulfite-toluidine blue (PST) procedure.
   (a) Prepare smear as described in Standard Methods for the Examination of Dairy Products, 10th edition, and dry with gentle warming on a suitable drying table.
   (b) Whole milk, cream, etc.—place smear in xylene for one minute, rinse in alcohol, place in fixative not less than 30 min., remove and air dry.

   Skimmilk, nonfat dry milk—place in fixative for not less than 30 min., remove and air dry. Preliminary defatting is unnecessary. Proper fixation is necessary to prevent sloughing in the next step. It also smooths and clears the milk film. An electric fan is helpful for rapid drying in this and subsequent steps.
   (c) Immerse for five min. in the periodic acid solution at room temperature, transfer to a container of absolute ethanol, remove and air-dry. The smear must be handled with care in this step.
   (d) Immerse for 5 min. in the bisulfite solution, remove and rinse thoroughly with water.
   (e) Immerse for 1 min. in the pH 4 dye solution, rinse lightly with water, and air dry.
2. Periodic acid–Schiff (PAS) procedure. Run as previously described by the author (4).
3. pH 4 Toluidine blue procedure (pH 4 TBO).
   (a) Fix 30 min. in alcohol–acetic acid mixture, dry.
   (b) Immerse in pH 4 dye solution, rinse in distilled water, and dry.

Bacterial cultures. Gram-positive and gram-negative species which are known to occur in milk were used in the heating experiments. The former group, obtained from stock cultures available in this laboratory, consisted of *Streptococcus faecalis*, *Lactobacillus arabinosus*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, and a *Staphylococcus* species (not further identified). The gram-negative organisms used were a coliform species (not further identified) from a stock culture and a pure culture of psychrophilic rods isolated from a container of milk stored in a refrigerator at 3-4° C.

TABLE 1

Effect of heating bacteria [a] in milk for 30 min. on direct microscopic clump counts

Bacteria	Stain	Direct microscopic clump count (10 fields)		
		Unheated	60° C.	100° C.
Streptococcus faecalis	PST [b]	1,344	1,312	1,388
	AOMB [c]	1,808	1,312	1,532
Lactobacillus arabinosus	PST	254	249	234
	AOMB	199	207	206
Lactobacillus casei	PST	69	73	90
	AOMB	49	41	57
Coliform (unidentified)	PST	640	666	757
	AOMB	685	505	33
Staphylococcus sp.	PST	47	52	51
	AOMB	55	44	37
Psychrophilic rods	PST	63	88	57
	AOMB	67	33	3
Leuconostoc mesenteroides	PST	268	289	279
	AOMB	221	131	265

[a] One milliliter of washed bacterial suspension from broth culture in 50 ml. of milk.
[b] Periodic acid–bisulfite–toluidine blue stain.
[c] Aniline oil–methylene blue stain.

The bacteria were grown in trypticase-soy broth (Difco) for 24-48 hr. at 32° C. (24° C. for psychrophilic rods) until turbidity indicated that nearly the maximum population had been reached, providing a uniform population of bacterial cells for experiments. The bacteria were spun down and washed three times in distilled water before use.

Heating experiments. The washed bacterial cells were suspended in distilled water for use in the heating experiments. Approximately 1 ml. of bacterial suspension was added to 50 ml. of whole milk for the experiments in which

TABLE 2

Stainability of psychrophilic rods by the periodic acid–bisulfite–toluidine blue procedure after heating in various media

Medium for heating	Staining after heating [a]
Water	Good
Milk	Poor
0.1 *M* phosphate buffer pH 5.9	Fair
0.1 *M* phosphate buffer pH 7.8	Poor
0.1 *M* phosphate buffer pH 9.0	Poor
0.25% NaCl	Poor
0.5% glycine	Good
0.5% bovine serum albumin (salt-free)	Good
0.5% ovalbumin	Good
Milk proteose–peptone (dialyzed)	Poor
0.25% $CaCl_2$	Fair
0.25% $MgCl_2$	Fair

[a] Heating time: 30 min. at 90-95° C.

TABLE 3

Staining behavior of different bacteria after heating[a] in various media

Bacteria	Stain	Unheated bacteria	Staining after heating in			Milk proteose–peptone
			Water	0.25% NaCl	Milk	
Streptococcus faecalis	pH 4 TBO[b]	Good	Good	Good	Poor	Very poor
	PST[c]	Excellent	Excellent	Excellent	Excellent	Excellent
	PAS[d]	Good	.....	.....	.....	.....
Staphylococcus sp.	pH 4 TBO	Good	Good	Good	Poor	Very poor
	PST	Good	Good	Good	Good	Good
	PAS	Good	.....	.....	.....	.....
Coliform sp.	pH 4 TBO	Good	Good	Poor	Poor	Poor
	PST	Good	Good	Poor	Poor	Poor
	PAS	Poor	.....	.....	.....	.....
Psychrophilic rods	pH 4 TBO	Good	Good	Poor	Poor	Poor
	PST	Good	Good	Poor	Poor	Poor
	PAS	Poor	.....	.....	.....	.....
Lactobacillus casei	pH 4 TBO	Good	Good	Poor	Poor	Poor
	PST	Excellent	Excellent	Excellent	Excellent	Excellent
	PAS	Good	.....	.....	.....	.....

[a] Heated 30 min. at 90-95° C.
[b] pH 4 TBO = pH 4 toluidine blue 0.
[c] PST = periodic acid–bisulfite–toluidine blue.
[d] PAS = periodic acid–Schiff.

heating in milk is indicated. For other experiments an equal volume of bacterial suspension and the medium in which it was to be heated were mixed to give final concentrations as shown in the tables.

For determination of nitrogen and phosphorus distribution (Table 4) between cells and supernatant, before and after heating, suspensions of washed bacterial cells were prepared and 10-ml. portions were pipetted into each of three test tubes. An equal volume of distilled water was added to Tubes 1 and 2, and Tube 3 was mixed with an equal volume of 0.5% NaCl to give a final concentration of 0.25%. Tube 1 served as the unheated control, whereas Tubes 2 and 3 were heated 30 min. at 90-95° C. After heating, the tubes were cooled in a water bath and the contents of all three were centrifuged down. The supernatant liquid was carefully decanted and the bacterial cells at the bottom of the tubes were resuspended in distilled water equivalent to the amount of supernatant decanted (20 ml.). A similar procedure was followed where proteose-peptone was used in place of NaCl. The bacterial suspension and the supernatant were analyzed for phosphorus by the method of Fiske and Subba Row (2) and for nitrogen using the Gunning-Arnold digestion followed by direct Nesslerization (3).

Milk-proteose-peptone (5). Whole milk was heated in a boiling water bath for 30 min. It was cooled, mixed with an equal volume of 2% acetic acid, and filtered. The clear filtrate was dialyzed at near 0° C. for 48 hr. against several changes of distilled water, then filtered again to remove a small amount of precipitate which formed.

RESULTS AND DISCUSSION

Table 1 shows the effect of heating on DMC's of various types of bacteria. The bacteria used were grown in broth culture, mixed with whole milk, and heated as indicated. The PST procedure and North's (AOMB) stain (7) were compared. The AOMB procedure did not effectively stain gram-negative rods that had been heated in milk. With the gram-positive bacteria, counts were in most cases about the same in unheated and heated samples, but the heated bacteria appeared shrunken and were difficult to see and identify. When the PST procedure was used, there was little change in either appearance of cells or DMC's after heating gram-positive organisms in milk. Gram-negative rods stained poorly after heating, but could be counted in about their original numbers.

Experiments were run to determine which components of milk were responsible for the loss of stainability in heated gram-negative rods (Table 2). Heating gram-negative psychrophilic rods for 30 min. in distilled water had little effect on their staining by the PST procedure, but stainability was gradually lost after prolonged heating. Various mineral salts, especially sodium chloride and phosphate buffers, caused considerable loss of stainability. Milk proteose–peptone, dialyzed to remove salts, were fully as effective as milk itself in causing loss of stainability. Glycine and salt-free protein solutions had no more effect on stainability than distilled water.

TABLE 4

Changes in nitrogen and phosphorus content of bacteria after heating[a] in water and 0.25% NaCl

Bacteria	Unheated				Heated in water				Heated in 0.25% NaCl			
	Mg. N/100 ml.		Mg. P/100 ml.		Mg. N/100 ml.		Mg. P/100 ml.		Mg. N/100 ml.		Mg. P/100 ml.	
	Cells	Supernatant	Cells	Supernatant	Cells	Supernatant	Cells	Supernatant	Cells	Supernatant	Cells	Supernatant
Staphylococcus sp.	0.60	0.24	0.080	0.038	0.56	0.20	0.063	0.045	0.32	0.24	0.067	0.06
Psychrophilic rods	2.6	0.6	0.26	0.04	2.3	0.9	0.21	0.12	1.7	1.8	0.08	0.23
Coliform	4.48	0.0	0.28	0.0	3.80	0.60	0.19	0.14	2.80	1.12	0.06	0.22
Streptococcus faecalis	4.20	0.24	0.92	0.07	3.60	0.44	0.83	0.10	3.36	0.56	0.78	0.23

[a] Heated 30 min. at 90-95° C.

The PST procedure operates both as nucleic acid and a polysaccharide stain (4). Table 3 shows the results obtained using relatively selective staining procedures; namely, the conventional periodic acid–Schiff (PAS) procedure (4) for polysaccharides, and toluidine blue buffered to pH 4 for nucleic acids (8). The latter procedure is based on the fact that this pH suppresses ionization of carboxyl groups, whereas phosphoric acid groups, such as are present in nucleic acids, being more acidic, remain as negative ions which will combine with the positive basic dye ions.

All types of gram-positive bacteria studied stained well by the PAS procedure, whereas gram-negative bacteria stained poorly. Staining with pH 4 TBO was good in all types of unheated bacteria but, after heating in milk or milk proteose-peptone, all types stained poorly, indicating loss of nucleic acids. Results were variable after heating in dilute sodium chloride solution. All types of heated bacteria that stained well with the PAS procedure, in general the gram-positive group, stained well by the PST method. Polysaccharides evidently are important in the staining of these gram-positive bacteria by the PST procedure.

Gram-negative rods, which stained poorly by the PAS procedure, stained poorly by the PST procedure after heat treatment. The results were similar to those obtained with pH 4 TBO, indicating that staining is dependent on the nucleic acid content. Loss of stainability might have resulted either from actual removal of nucleic acids from the cells or from some change caused by heating which made them unable to take up the stain. A series of experiments was run to clarify this point.

TABLE 5

Changes in nitrogen and phosphorus content of *Streptococcus faecalis* after heating[a] in dialyzed milk proteose–peptone

	Mg. N/100 ml.		Mg. P/100 ml.	
	Cells	Supernatant	Cells	Supernatant
Proteose-peptone solution	.....	6.00	.....	0.135
Unheated = 1 part bacterial suspension / 1 part distilled H_2O	2.08	0.44	0.231	0.030
Heated = 1 part bacterial suspension / 1 part proteose-peptone	2.16	3.60	0.142	0.210
Net change (corrected for proteose-peptone)	+0.08	+0.16	−0.089	+0.118

[a] Heated 30 min. at 90-95° C.

Tables 4 and 5 show the effect of heating on the nitrogen and phosphorus content of the bacteria. The two types of gram-negative rods studied lost approximately 75% of their phosphorus content on heating in dilute sodium chloride solution and this is associated with loss of stainability for nucleic acids, as shown in Table 3. The *Staphylococcus* and *Streptococcus* species lost little nitrogen and phosphorus on heating in dilute sodium chloride and like-

wise showed little loss of stainability for nucleic acids under these conditions. None lost much nitrogen and phosphorus after heating 30 min. in distilled water, nor did they lose stainability for nucleic acids under these conditions. *S. faecalis*, on the other hand, does lose its stainability for nucleic acids on heating in milk proteose–peptone, and considerable extraction of phosphorus takes place (Table 5).

An analysis of an extract of the psychrophilic rods after heating 30 min. in dilute sodium chloride showed considerable amounts of ribonucleic acid using the orcinol reaction (6) and some deoxyribonucleic as shown by the diphenylamine reaction (6). The O.D. 280/260 was approximately 0.5, which is about that of a pure nucleic acid solution (10), indicating that the extract is practically protein-free.

The data from this series of experiments indicate that the PST procedure should be entirely satisfactory for staining gram-positive bacteria in heated milk. It does not stain gram-negative bacteria well in heated milk, but they can be seen well enough to be counted, which is not the case with the AOMB stain. Table 6 shows the results of DMC's made on some samples of nonfat dry milk, in order to compare the performance of the PST and the AOMB procedures on commercial samples. Total counts using the PST procedure ranged from about the same to nearly double those obtained using the AOMB stain. The number of rods counted using the PST stain was always higher, sometimes by a considerable amount, than was found using the AOMB stain. These data agree with what might be predicted from the experiments using pure cultures.

It was hoped originally that this study might lead to improved staining methods for gram-negative rods. Attempts to add nucleic acid to the cells after heating have thus far not been successful and it has not yet been possible to improve on the basic PST procedure.

TABLE 6

Comparison of the aniline oil–methylene blue (AOMB) and the periodic acid–bisulfite–toluidine blue (PST) stains for direct microscopic clump counts (DMC's) of bacteria in commercial nonfat dry milk samples

Process [a]	DMC using PST stain (millions/gram)		DMC using AOMB stain (millions/gram)	
	Total count	Rod count	Total count	Rod count
R	64	4	45	3
HH	171	24	96	8
HH	139	20	115	12
HH	66	18	37	4
HH	64	12	26	5
HH	28	6	26	2
MH	142	16	74	1
MH	40	2	22	1
LH	88	13	39	1
LH	55	8	20	1

[a] R = Roller.
LH = Low-heat spray-dried.
MH = Medium-heat spray-dried.
HH = High-heat spray-dried.

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BACTERIOLOGICAL EVALUATION OF MANUFACTURING-GRADE BULK-TANK MILK [1, 2]

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SUMMARY

Standard, thermoduric, and psychrophilic counts were made on 701 bulk-tank milk samples of manufacturing grade. These samples were obtained from 267 Iowa producers at all seasons of the year. Plates for standard and thermoduric counts were incubated at 32° C. Direct microscopic clump counts were made on 586 samples.

The SPC of 37.7% of the samples exceeded 1,000,000 per milliliter, as compared to 26.5% at this level for the psychrophilic plate count results. Thermoduric counts in excess of 10,000 per milliliter were obtained on 43.6% of the samples.

The direct microscopic clump count was considerably less than the SPC on many samples. Of the 245 samples out of 586 comparisons where the results of these two counts failed to agree, all but 16.3% of the samples had high counts of psychrophilic or thermoduric bacteria. Most psychrophilic bacteria stained readily, but many appeared to grow in clumps. The clumps probably were broken up during preparation of dilutions for plating. Thermoduric organisms apparently did not readily take up the stain, although they were not specifically tested.

Increasing use of farm bulk tanks for handling manufacturing-grade milk means more of this milk is being cooled to 40° F. and below. Much of this milk is picked up every other day, or three times a week. Thus temperature, time of holding, and milk storage equipment are different from those commonly encountered in handling such milk in cans. The microflora which develops in such milk might be expected to be different from that of milk held for shorter periods of time at higher temperatures in cans.

Johnson *et al.* (6), Atherton (2), and Pearson (9) are among those who have studied the bacteriological aspects of handling Grade A milk in both bulk tanks and cans. They indicated some improvements in bacterial counts for the samples as a whole when a shift was made to bulk tanks, but differences were seldom large. Some producers actually delivered milk with higher counts after converting to bulk tanks.

In the present study, the quality of manufacturing-grade bulk-tank milk was studied where production conditions on the farms, in many cases, did not approximate Grade A requirements. The usefulness of and relationships between

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the direct microscopic clump count, the plate count, and the counts of thermoduric and psychrophilic bacteria were studied.

METHODS

The 701 manufacturing-grade bulk-tank samples examined were from 267 producers. These represented deliveries to four Iowa dairies during all seasons of the year. The samples were collected at the farm by the senior author. Collection and subsequent laboratory examination were in accordance with procedures outlined in the tenth edition of Standard Methods for the Examination of Dairy Products (1). Standard plate count, thermoduric, and psychrophilic counts were determined. Plates for the standard and thermoduric counts were incubated at 32° C. Plates for the psychrophilic count were incubated at 5° C. for seven days.

Direct microscopic clump counts were made on 586 of the 701 samples. The staining technique developed by Levowitz and Weber (7) was used.

Individual colonies from plates for psychrophilic counts were picked onto plate count agar (PCA) slants, grown at 23° C. for 24 hr., and restreaked for purification. Cultures to be characterized were picked from a 24-hr. PCA slant into a carbohydrate-free broth and grown at 23° C. for 20 hr. A broth culture was used for inoculation purposes. The methods and media used for identification were suggested mainly by the Manual of Microbiological Methods (10).

The staining ability of representative psychrophilic cultures was determined on cultures grown in raw milk. Milk of known low bacterial content was obtained by aseptic means from the Iowa State University dairy farm bulk tank and immediately taken to the laboratory in a refrigerated container. One drop of a 20-hr. broth culture was added to 100 ml. of milk in a 6-oz. prescription bottle and thoroughly mixed. Initial bacterial counts were made on the inoculated milk and an uninoculated control by plating on PCA and incubating the plates at 23° C. for 72 hr. The inoculated and control bottles were incubated at 5° C. for 72 hr. The bottles were gently mixed each day. After incubation, plate counts were made, using incubation at 23° C. for 72 hr. Smears for the direct microscopic clump counts were prepared as they were for the usual raw milk samples.

RESULTS

Results of the standard plate counts on the 701 milk samples showed that 37.2% of the total had counts below 200,000 per milliliter, 25.1% fell between 200,000 and 1,000,000 per milliliter, and 37.7% were in excess of 1,000,000 per milliliter.

Psychrophilic plate count results on the milk samples revealed 59.5% of the total had counts less than 200,000 per milliliter, 14.0% were between 200,000 and 1,000,000 per milliliter, and 26.5% exceeded 1,000,000 per milliliter.

The 79 cultures of bacteria isolated from the plates for psychrophilic counts included 51 *Pseudomonas*, nine *Achromobacter*, eight *Alcaligenes*, seven *Flavobacterium*, three *Aerobacter*, and one *Escherichia*, using the criteria of Bergey's Manual (3) for differentiation. Fifty-six of the isolates, or 70.9% of the total,

showed evidence of lipase production. These included 42 *Pseudomonas*, four *Achromobacter*, eight *Alcaligenes*, one *Flavobacterium*, and one *Aerobacter*. Twenty-eight of the isolates, or 35.5% of the total, failed to grow at 35° C. These included 14 *Pseudomonas*, six *Achromobacter*, five *Flavobacterium*, and three *Alcaligenes* strains. All others were able to show visible growth on PCA slants within 48 hr.

Thermoduric counts on the 701 laboratory-pasteurized samples disclosed that 56.4% of the total had counts below 10,000 per milliliter and 29.1% had counts between 10,000 and 100,000 per milliliter, and 14.5% exceeded 100,000 per milliliter.

TABLE 1

Comparisons of direct microscopic counts with standard plate counts on bulk-tank milk

Total samples	DMC and SPC not in agreement [a]	DMC and SPC were not in agreement when:							
		PPC high [b]		TPC high [c]		PPC and TPC high		Neither PPC nor TPC high	
	(No.)	*(%)*[d]	*(No.)*	*(%)*[e]	*(No.)*	*(%)*[e]	*(No.)*	*(%)*[e]	*(No.)*	*(%)*[e]
586	245	41.8	103	42.1	69	28.1	33	13.5	40	16.3

[a] Standard plate count (SPC) at least twice as high as direct microscopic clump count (DMC).
[b] Psychrophilic plate count 75% of or higher than the standard plate count (SPC).
[c] Thermoduric plate count (TPC) in excess of 30,000/milliliter.
[d] Per cent of total samples not agreeing.
[e] Per cent of the 245 samples not agreeing.

Results of the standard plate count and the direct microscopic clump count were compared on 586 milk samples. Table 1 summarizes the data. None of the samples had direct microscopic counts that exceeded the standard plate count to any significant extent. A lack of agreement between the two methods was considered to exist where the standard plate count was at least twice as high as the direct microscopic count. Of the 245 samples (41.8% of the total) showing lack of agreement, 103, or 42.1%, had psychrophilic counts that were 75% or more of the standard plate count. Sixty-nine samples, or 28.1%, had thermoduric counts that were in excess of 30,000 per milliliter. Counts at this level or above constituted, in this study, one explanation for poor agreement between the standard plate count and direct microscopic count. While the 30,000-per-milliliter level might not be considered adequate to markedly influence the direct microscopic count, it would be assumed that many more thermoduric bacteria, slightly less heat-tolerant than the survivors of the laboratory-pasteurization heat treatment, were present in the raw sample. It is undoubtedly this large number which contributes to the discrepancy in the standard plate count and direct microscopic count. Thirty-three samples, or 13.5%, had both high thermoduric and high psychrophilic counts and 40, or 16.3%, had neither a high thermoduric nor a high psychrophilic count.

Table 2 presents the results of the direct microscopic clump count on 31 psychrophilic cultures grown in low-count raw milk at 5° C. for 72 hr. All

psychrophilic cultures grown in raw milk had higher plate counts than direct microscopic clump counts. Cultures with a wide difference between the results of the two tests tended to grow in clumps, with one exception. The cells of Culture 48 were difficult to see in the stained preparation. All other cultures appeared to stain well enough for relatively easy observation of the cells.

DISCUSSION

The relatively poor bacteriological quality of many manufacturing grade bulk-tank milk samples examined indicates that the bulk tank is far from being

TABLE 2

Direct microscopic counts on several psychrophilic cultures grown in raw milk

Culture number and genus	Plate count after 3 days at 5° C.[a]	Direct microscopic clump count[b]	Predominant cell distribution
Control	4T[c]	40T	
2 Pseudomonas	40M[d]	16.0M	Clumps
5 Pseudomonas	21M	5.0M	Clumps
8 Achromobacter	67M	54M	Pairs
10 Flavobacterium	1.5M	440T	Small clumps
12 Achromobacter	320T	60T	
15 Pseudomonas	34M	10M	Clumps
17 Alcaligenes	250T	220M	
19 Alcaligenes	300T	220M	Pairs
21 Aerobacter	27M	16.0M	Pairs and singles
23 Pseudomonas	25M	24.0M	Pairs and singles
29 Pseudomonas	60M	12.0M	Pairs and clumps
31 Achromobacter	6.8M	1.6M	Clumps and pairs
33 Achromobacter	12M	8.9M	Pairs and singles
36 Pseudomonas	16M	2.6M	Clumps
38 Flavobacterium	7.8M	4.4M	Small clumps
40 Pseudomonas	8.3M	780T	Clumps
42 Achromobacter	560T	260T	Small clumps
44 Escherichia	700T	420T	Small clumps
46 Pseudomonas	16M	3.0M	Clumps
48 Flavobacterium	24M	11.0M	(Stained lightly)
49 Pseudomonas	65M	16.0M	Clumps
52 Alcaligenes	14M	240T	Clumps
54 Pseudomonas	26M	4.4M	Clumps
56 Pseudomonas	100M	23.0M	Pairs, individuals, clumps
58 Pseudomonas	19M	4.0M	Clumps
61 Aerobacter	6.3M	1.6M	Clumps
62 Achromobacter	8M	1.1M	Clumps
63 Pseudomonas	42M	25.0M	Pairs and individuals
67 Pseudomonas	39M	6.7M	Small clumps
69 Pseudomonas	60M	13.0M	Clumps
75 Pseudomonas	75M	72.0M	Pairs and individuals

[a] One drop of a 20-hr. broth culture in 4 oz. of raw milk before incubation. Plates incubated at 23° C. for three days.

[b] Levowitz-Weber staining technique, clumps counted.

[c] T = Thousand.

[d] M = Million.

the complete answer to producing and handling quality milk. An inadequate job of cleaning the bulk tank undoubtedly accounts, to a large extent, for many high bacterial counts obtained. The high proportion of psychrophilic bacteria is noteworthy. Because the bulk tank is kept at refrigeration temperature most of the time, the bacteria growing in that environment would be expected to be predominantly psychrophilic. The time between emptying and refilling would not be a long enough interval at a higher temperature that one would expect the character of the microflora to change to any great extent. With the factors of time and warmer atmospheric temperatures, the psychrophilic organisms might increase in numbers very rapidly in the unwashed or poorly washed tank. In spite of the low holding temperatures of the milk subsequently placed in the bulk tank, these residual organisms would slowly increase in numbers and frequently make up a large proportion of the microflora of the milk.

When the producer fails to clean and sanitize his milking equipment which is held at milk-house temperatures between uses, opportunity exists for thermoduric organisms to grow on the equipment, especially during the warmer months. This equipment would become a prime source for contaminating the milk. If a combination of poor care of the bulk tank and of the milking and milk handling equipment prevailed, the microflora of the milk might include large numbers of both psychrophilic and thermoduric bacteria.

The primary factor responsible for the wide discrepancies between plate counts and direct microscopic counts appears to be the tendency for many of the psychrophilic organisms to grow in clumps in raw milk. The necessary shaking of the dilutions during plating procedures may break a bacterial clump into several portions, each of which may produce a colony. The psychrophilic cultures which grew as single cells and pairs had microscopic clump counts comparable to the standard plate count. The comparability of these two tests when the milk contained a large proportion of psychrophilic bacteria would depend to a large degree on whether the majority of the psychrophilic bacteria grew in clumps or as individual cells and/or pairs. The possibility also exists that some of the psychrophilic bacteria stain so poorly they are not counted under routine conditions.

Over 41% of the samples that were not classified properly by the direct microscopic clump count, as judged by the standard plate count, had thermoduric plate counts in excess of 30,000 per milliliter. Although the staining ability of thermoduric isolates was not studied, many thermoduric organisms apparently do not stain readily and, thus, are hard to enumerate. Little (8) found that the microscopic count was not accurate in determining the bacteriological quality of raw milk when the samples contained large numbers of thermoduric bacteria.

The direct microscopic clump count appears to be of limited value for estimating the bacterial population of manufacturing-grade bulk-tank milk where the predominant microflora could be made up of large numbers of psychrophilic or thermoduric organisms. Jezeski *et al.* (5) and the Committee on Applied Laboratory Methods (4) previously found the direct microscopic count

not as efficient as the standard plate count in picking out unsatisfactory raw milk samples with counts exceeding 200,000 per milliliter.

The plate count with incubation at 32° C., instead of 35° C., appears the procedure of choice for bacteriological classification of bulk-tank raw milk, because the lower temperature permits growth of more psychrophilic bacteria. Modifications of the plate count procedure to make it more applicable to plant routine might prove desirable.

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AN IMPROVED NONSELECTIVE CULTURE MEDIUM FOR RUMINAL BACTERIA AND ITS USE IN DETERMINING DIURNAL VARIATION IN NUMBERS OF BACTERIA IN THE RUMEN

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SUMMARY

An improved rumen fluid agar medium was developed that permitted the growth of about double the numbers of bacteria from ruminal contents grown in the medium previously used. It allowed much larger numbers of colonies to be counted and isolated with greater ease from a given volume of medium and after longer incubation periods. The main modifications made were the use of rumen fluid clarified by centrifugation, reducing the concentration of the sugar energy sources (glucose and cellobiose) and inclusion of soluble starch as an additional energy source, and changing the reducing agent from cysteine to a combination of cysteine and sodium sulfide. Many other modifications in the medium were tested and found to be without effect or detrimental to colony counts. Experiments indicate that about the same range of species of bacteria are cultured in the improved medium as in the old, except that the improved medium allows the growth of bacteroides strains that utilize starch but not glucose or cellobiose.

Significant differences were shown in nonselective viable counts of ruminal bacteria obtained from samples of rumen contents collected at different times during the day from a cow fed alfalfa hay or a grain mixture once a day, and there were also differences in counts due to the ration.

It was previously concluded that agar culture media for the nonselective isolation and enumeration of ruminal bacteria should contain rumen fluid, a relatively high level of bicarbonate and/or CO_2, a neutral or slightly acid pH and a reducing agent to give a low E_h [see (2) for discussion and references]. Also, most media have contained added carbohydrates and minerals and, in some cases, additional crude sources of growth factors.

Although various workers have attempted to obtain improved media by replacing or augmenting rumen fluid with various extracts and hydrolysates commonly used in bacteriological media with little apparent success, a relatively small amount of work has been directed toward improvement of the rumen fluid medium per se. This medium, as presently used in several laboratories, contains unclarified rumen fluid which often contains some particulate matter, and the level of carbohydrate added is such that large colonies, spreading, and gas formation are a great disadvantage to counting colonies or making isolations, especially if the cultures are incubated for more than three days.

The present study was initiated in an attempt to improve the rumen fluid agar medium for nonselective isolation and enumeration of ruminal bacteria, both from the standpoint of ease of counting colonies and isolation of strains and of the possibility of obtaining higher total counts. In addition, a brief study was done (using the improved medium) to determine whether significant

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differences in colony counts could be obtained from ruminal contents obtained from an animal at different times after feeding or when different rations were fed.

METHODS

Preparation of media. Anaerobic techniques and the method of preparing media for roll tubes were as previously described (3), with the following exceptions: Gases used to maintain anaerobic conditions were passed through a column of hot reduced copper filings to remove traces of O_2. Rumen fluid additions were made before boiling the medium to dissolve agar, and reducing agents were added to individual tubes of medium shortly before it was melted prior to inoculation.

The composition of the media used is shown in the results. The various solutions added to media were prepared as follows. Resazurin solution and Minerals 1 and 2 (reagent-grade salts) were as previously described, except that the mineral concentrations were doubled. Na_2CO_3 (reagent grade) and L-cysteine · HCl · H_2O (reagent grade) solutions were as previously described, being prepared and stored under CO_2 gas, but were prepared in 8% (w/v) and 2.5% (w/v) concentrations, respectively. $Na_2S \cdot 9H_2O$ (reagent-grade) solution was prepared by dissolving 2.5 g. in water to which one drop of 10% NaOH was added. The solution was made to 100 ml. with distilled water, boiled under N_2, autoclaved 10 min. at 15 lb. in rubber-stoppered flasks with N_2 gaseous phase, tubed in appropriate amounts under N_2, and stored in the refrigerator. In experiments where a mixture of sulfide and cysteine was used, they were prepared as a single solution by the same procedure used for preparation of the sulfide alone, except that enough NaOH was added to the cysteine solution to bring it to pH 11 before adding the sulfide. The addition of alkali and use of N_2 in place of CO_2, as well as the addition of reducing agents after media were tubed, were precautionary measures taken to minimize loss of sulfide as H_2S during preparation of media.

Clarified rumen fluid No. 1 (CRF1) was prepared as follows: Rumen fluid, collected as previously described (3), was centrifuged at approximately 25,000 $\times$ g for 20 min. CRF 2 was prepared in the same manner, except that the rumen fluid was briefly boiled under CO_2 and then autoclaved under CO_2 for 5 min. at 15 lb. before centrifuging. When rumen fluid, CRF 1, and CRF 2 were compared, they were always prepared from the same batch of rumen fluid. In later work, where CRF 2 was used, the rumen fluid was autoclaved under CO_2 in flasks shortly after collection and stored in the refrigerator. It was centrifuged just before media were prepared.

Dry materials weighed out and added directly to media before autoclaving included soluble starch and sodium citrate · $2H_2O$ (both reagent grade), cellobiose (Eastman), and glucose (Bacto).

Inoculation and counting procedure. The procedure used for sampling ruminal contents and inoculating media was varied from that previously used (3), in the following ways: The dilution solution was changed to that of Bryant and Robinson (4). Twenty grams, rather than 20 ml. of rumen contents were diluted with 180 ml. of dilution solution for the 10^1 dilution. After mixing this

dilution in the Waring blendor, tenfold serial dilutions (1 ml. in 9 ml. of dilution solution) were made, usually through the 10^6 dilution. Then, further appropriate dilutions were made so that 0.1 ml. of the dilution inoculated into the medium would be appropriate for counting colonies. Usually, 1.4 ml. of the 10^6 dilution was diluted to 7 ml. to make a 2×10^7 dilution in a Wassermann tube. The Wassermann tube was used for the final dilution to facilitate removal of solution with a 2-ml. Cornwall syringe and a 2-in. No. 18 needle, which was used to inoculate tubes of medium by piercing the rubber stopper, according to the method of Smith and Hungate (14). Five tubes of each medium at each dilution (usually only one) were inoculated.

Colony counts used in statistical analyses were averages of five tubes counted after three and seven days of incubation at 37° C., unless otherwise stated. All colonies observable, using the Quebec colony counter plus questionable colonies that could be confirmed using a stereoscopic microscope were counted.

Unless otherwise stated, rumen contents used as inoculum was collected at 11 A.M. from one Holstein cow fed twice daily at the recommended maintenance level on a ration of alfalfa hay (79%) and a grain mixture. At times, the hay was rather poor quality and contained some grass.

When experiments were done to see if the cultural methods used would show significant differences in colony counts from samples of rumen contents collected at various times after feeding, a Holstein cow was placed on a recommended maintenance level of good quality alfalfa hay or grain mixture (15% crude protein) fed at 7:00. Water was available at 5:00 to 7:00 and 13:00 to 14:00. Samples were collected 11 and 32 days after grain feeding was initiated and 30 and 39 days after hay feeding was started.

Statistical analyses were carried out by analysis of variance and, at times, by Duncan's multiple range test (10).

RESULTS AND DISCUSSION

Effect of time of incubation on colony counts. Results indicated that colony counts increased at least through 14 days of incubation; however, 83% of the 14-day-colonies had appeared by the seventh day (Table 1). Analysis of data

TABLE 1

A comparison of colony counts obtained in media [a] containing whole rumen fluid or rumen fluids clarified by two procedures

	Colony counts [b]					
	40% Rumen fluid			20% Rumen fluid		
Incubation	3 days	7 days	14 days	3 days	7 days	14 days
Treatment						
RF	4.0	6.3	7.6	2.3	3.4	4.4
CRF 1	5.4	7.4	9.4	4.5	5.3	6.2
CRF 2	5.3	7.1	7.9	3.5	5.0	6.4

[a] In addition to components listed above, the media contained 2% (w/v) agar, 0.0001% (w/v) resazurin, 7.5% (v/v) each of Minerals 1 and 2, 0.05% (w/v) each of glucose and cellobiose, 0.2% (w/v) of Na_2CO_3, 0.05% (w/v) of cysteine · HCl · H_2O, and was in equilibrium with 50% CO_2—50% H_2 gas.

[b] Values are averages of two experiments. Inoculum was 2×10^{-5} g. of rumen contents.

on three- and seven-day counts from eight different groups of experiments (16 individual experiments) showed that three-day counts averaged 67% (range of 54-75%) of the seven-day counts. The difference was highly significant (0.01 level of probability) in seven of the eight experiments and in none was there a significant time × treatment interaction.

Clarification and levels of rumen fluid. Analysis of variance of data of experiments shown in Table 1 indicated no significant difference between counts in media containing rumen fluid or the two clarified rumen fluids. Similar results were obtained in two experiments where CRF 2 and rumen fluid were compared in media containing the same ingredients as those of Table 1 and in media containing double the amount of Na_2CO_3 and a 100% CO_2 gaseous phase.

There likewise was no apparent difference between the media containing the 100% and 50% CO_2 gaseous phases with corresponding levels of Na_2CO_3.

The results on clarified rumen fluid seem to differ from those of McNeill *et al.* (11), who found that centrifuging out the cells in rumen fluid incorporated into media caused a reduction in colony counts. No explanation for this difference is apparent.

Data in Tables 1 and 2 indicate that colony counts were significantly higher with 40% than with 20% rumen fluid (0.01 level of probability), whereas increasing the level to 60% (Table 2) had no apparent effect. There was not a significant level of rumen fluid × mineral level interaction (Table 2).

TABLE 2

A comparison of colony counts [a] obtained in media [b] containing different levels of CRF 2 and minerals

Mineral level *(%)*	0	3.75
Level of CRF *(%)*		
20	9.2	8.5
40	12.4	10.7
60	12.1	11.7

[a] Values are averages of the seven-day counts of four experiments. Inoculum was 2×10^{-8} g. of rumen contents.

[b] In addition to the components listed above, the media contained the same ingredients as those listed in Table 1, Footnote a, except that the reducing agent was 0.025% each of cysteine · HCl · H_2O and $Na_2S \cdot 9H_2O$.

Reducing agents and catalase. A review of the literature (2) revealed little information on the effect of kinds and levels of reducing agents on colony counts from rumen contents. However, materials such as ascorbic acid, sodium formaldehyde sulfoxylate, thioglycolate, palladium black-hydrogen, dithionite, and sulfide have been found to be more or less unsatisfactory for one or more species of ruminal bacteria in liquid culture. In general, cysteine appeared to be the best reducing agent. However, in a study of ruminococci (1), Na_2S was found to be superior to cysteine; and, although Na_2S was found to be inferior to cysteine for a strain of *Butyrivibrio* (7), the levels used were higher than necessary in the present authors' hands.

As sulfide is a common constituent of rumen fluid, experiments were performed to compare it with cysteine as a reducing agent for colony counts (Table 3). The data show that the lower level of sulfide alone or in combination with cysteine resulted in higher colony counts than cysteine only. Both reducing agents at the lower level were used in later experiments.

TABLE 3

Colony counts of media [a] containing different levels and combinations of cysteine and sulfide reducing agents

Concentration		Colony counts [b]	Statistical significance [c]
Cysteine · HCl · H_2O	Na_2S · $9H_2O$		
0	.025	23.8	a
.025	.025	21.1	a, b
.05	.025	19.1	a, b, c
0	.05	17.6	b, c, d
.025	.05	16.3	b, c, d
.025	0	14.1	c, d
.05	.05	13.3	d
.05	0	12.4	d
0	0	0	e

[a] In addition to the reducing agents shown above, the media ingredients included 40% of CRF 2 and the components listed in Table 1, Footnote a, except that 3.75% each of Minerals 1 and 2 and 0.3% of Na_2CO_3 were used.

[b] Averages of two experiments. Inoculum was 2×10^{-8} g. of rumen contents.

[c] Colony count followed by letter *a* is significantly different (0.05 level of probability) from those means not having *a*; those followed by *b* are significantly different from those not having *b*, etc.

Because of the known effect of catalase on growth of clostridia near the surface of media exposed to oxygen (9), levels of approximately 0, 25, 50, and 100 units of catalase per 9 ml. of medium were added to media just before inoculation. Catalase had no effect on colony counts under the present anaerobic conditions.

Mineral concentrations and different batches of rumen fluid. Many experiments were carried out in which different concentrations of Minerals 1 and 2 (0, 3.75, and 7.5% of each), of Na_2CO_3 (0.1, 0.2, 0.3, and 0.4%), and batches of CRF 2, collected at different times or from different cows, were compared. The 0.1% level of Na_2CO_3 was inferior to the others, probably because of the lower pH of the medium. Although in some individual experiments the other levels of Na_2CO_3 and all levels of minerals might show differences, data from replicated experiments did not indicate a distinct advantage of one combination. The different batches of CRF 2 did not result in significantly different colony counts; and there were no significant interactions between any of the following: level of Na_2CO_3 (0.2 and 0.3%), level of Minerals 1 and 2 (0 and 3.75%), reducing agent (0.025% cysteine · H_2O · HCl or Na_2S · $9H_2O$), and batch of CRF 2. It seemed probable that different results in replicated experiments were due to unknown factors involved in the preparation of media.

In other experiments, the concentration of $(NH_4)_2SO_4$, usually present in Mineral 2, added to media was varied (0.09, 0.045, 0.023, and 0%) without any

effect on colony counts, and the addition of a chelating agent, sodium citrate · $2H_2O$, in concentrations of 0, 15, and 30 mg. % to media containing 0 or 3.75% of Minerals 1 and 2 had no effect. Sixty milligrams per cent inhibited counts (0.01 level of probability).

Carbohydrate levels. In preliminary experiments it was found that even 0.1% each of glucose and cellobiose was too high a concentration of carbohydrate for satisfactory colony counts after seven days of incubation, because of large colonies, spreading, and gas. A replicated experiment was performed using 0, 0.025, and 0.05% each of glucose and cellobiose in the medium (otherwise, the medium ingredients included 40% of CRF 2 and the components listed in Table 1, Footnote a, except that 0.025% each of cysteine · HCl · H_2O and Na_2S · $9H_2O$ and 3.75% each of Minerals 1 and 2 were used). There were only small differences in colony counts—the 0.025% concentration being significantly higher than 0% (0.05 level of probability). Of greater significance was the fact that with no added sugar many colonies after both three and seven days of incubation were so small that difficulty wauld be encountered in attempting their isolation. The 0.025% concentration drastically limited size of colonies, but allowed them to reach sizes large enough for relatively easy subculture after only three days of incubation.

Because colony counts of certain nonruminal species of bacteria can be increased by adding soluble starch to the growth medium, a study was made of the effects of 0, 0.025, 0.05, and 0.10% of soluble starch in the above medium containing 0.025% of each sugar. Results of the experiment replicated four times indicated no significant difference. As one species of ruminal bacteria, *Bacteroides amylophilus*, is known to utilize starch or maltose but not glucose or cellobiose (8), it was decided to incorporate 0.05% of soluble starch in the medium. More recent unpublished data by the authors indicate that organisms presumptively identified with this species are among the predominant ruminal bacteria grown on this medium. It was never isolated before starch was added to the medium.

Direct comparisons of the old and the new media. The composition of the Medium (98-5) finally selected for obtaining colony counts from rumen contents was as follows: glucose and cellobiose, 0.025% (w/v) of each; soluble starch, 0.05% (w/v); agar, 2.0% (w/v); resazurin solution, 0.1% (v/v); Minerals 1 and 2, 3.75% (v/v) of each; Na_2CO_3 solution, 2.5% (v/v), and cysteine-Na_2S solution, 1% (v/v). The medium was in equilibrium with 50% CO_2—50% H_2 gas.

Results in Table 4 show that Medium 98-5 supported the growth of about double the number of colonies grown in the medium previously used (RGCA). It was also indicated that doubling the level of CO_2 and Na_2CO_3 in the new medium did not significantly affect colony counts.

To determine if Medium 98-5 supported growth of similar groups of bacteria to those grown in the RGCA medium, colonies were transferred to slants from roll tubes of both media, inoculated with the same batch and dilution of rumen contents, after three and seven days of incubation. The strains obtained were

TABLE 4

A comparison of colony counts obtained in the old rumen fluid medium (RGCA) and the new CRF medium (98-5) with the 50% CO_2—50% H_2 gaseous phase and 0.2% Na_2CO_3 or modified to contain 100% CO_2 and 0.4% Na_2CO_3

Medium	Colony count [a]			Statistical significance [b]
	3 day	7 day	Average	
RGCA	14.5	20.5	17.5	a
98-5	30.5	37.0	33.8	b
98-5 with 100% CO_2	32.5	41.5	37.0	b

[a] Values are averages of two experiments with separate batches of media and inocula (3×10^{-8} g.) from different animals.

[b] See Table 3, Footnote c, except that differences were significant at the 0.01 level of probability.

grouped on the basis of colony type and morphology as determined in wet mounts and gram stains (2,3). The following number of strains of different groups were obtained (Medium 98-5 and RGCA, respectively): total, 80 and 84; gram-negative rods similar to *Bacteroides ruminicola*, *B. succinogenes*, and *B. amylophilus*, 22 and 28; *Selenomonas*-like, 5 and 3; *Lachnospira*-like, 0 and 1; *Borrelia* sp., 1 and 1; other motile curved rods such as *Butyrivibrio* sp., 20 and 29; *Succinimonas*-like, 1 and 0; *Ruminococcus*-like, 7 and 9; Streptococcus-like, 4 and 1; others, 20 and 12.

The results suggested no substantial difference in the groups of organisms isolated; and more recent unpublished work, in which certain nutritional requirements and morphology were determined for many strains isolated using Medium 98-5, indicated that all of the above groups were isolated except *Streptococcus*-like and *Succinimonas*-like strains, and *Eubacterium ruminantium*-like strains; and others recognized but not yet named or studied in detail were also isolated. The flora grown with the new medium appears to be at least as diverse as that grown in the RGCA medium.

Another advantage of Medium 98-5 over the RGCA medium was that colony counts of more than 200 per roll tube (9 ml. of medium) could be counted with ease and, where colony counts from tubes inoculated with different dilutions of the same sample were compared, there appeared to be little inhibition of colony development in tubes containing up to 200 colonies. In the RGCA medium, accurate counts from roll tubes containing 60-80 colonies are often difficult to obtain.

To determine if the anaerobic mineral solution used to dilute rumen contents prior to inoculating media was satisfactory, two experiments were done, in which three subsamples of the contents were diluted in the anaerobic mineral solution (No. 15) and three were diluted in the same solution but with 40% of CRF 2 added. The order of inoculation from the two solutions was No. 15, No. 15+CRF, No. 15, etc., and was reversed in the second experiment. Average colony counts in Medium 98-5 were 17.3 and 15.2 for the anaerobic mineral solution and the CRF 2 solution, respectively. The difference was slight but significantly different at the 0.05 level of probability. There was no significant difference due to the order of inoculation. The results suggested that the

anaerobic mineral solution was quite satisfactory and it was easier to work with, because the CRF solution tended to foam during shaking.

Variation in numbers of bacteria in the rumen as revealed by colony counts in Medium 98-5. Data in Table 5 show that significant differences occurred in colony counts obtained from samples from ruminal contents collected from a cow at different times during the day, and analysis of colony counts of samples collected at corresponding times for the two rations indicated the grain supported higher counts (0.05 level of probability) than the hay. No ration × time interaction was found.

TABLE 5

A comparison of colony counts obtained from samples of rumen contents collected at different times of the day from a cow fed once a day at 7:00

Hay ration			Grain ration		
Time	Colony count [a]	Statistical significance [b]	Time	Colony count [a]	Statistical significance [b]
	(billions per gram)			*(billions per gram)*	
9:00	1.03	a	8:00	2.22	a
7:00	1.65	b	7:00	4.15	a, b
11:00	2.37	c	9:00	4.22	a, b
15:00	2.81	c, d	10:00	5.10	a, b
13:00	2.94	c, d	5:00	6.22	b, c
17:00	3.01	d	23:00	6.82	b, c, d
19:00	3.21	d	17:00	9.58	c, d, e
			15:00	9.68	c, d, e
			13:00	10.48	d, e
			11:00	10.98	e

[a] Counts are means of two experiments run on separate days, which included means of counts made after three and seven days of incubation of five tubes inoculated with a single dilution of rumen fluid.

[b] See Table 3, Footnote c.

The data showing the change in counts through the day and the great difference in counts found on different days when the animal was on grain are presented in graphic form in Figure 1. It is evident that a drop in count occurred between 5:00 and 7:00 which corresponded with the time of first water intake of the day. Then, there was a further drop during the first 1-2 hr. after feeding, possibly due to dilution of the contents with feed and saliva. The numbers then rapidly increased for 2-4 hr., then declined (grain ration) or plateaued (hay ration) to levels approaching the numbers found in the early morning before water and feed were consumed.

Although precise comparisons are useless because of differences in animal species, rations, and sampling procedures, the present trends in counts through the day show some similarities to trends in direct microscopic counts obtained in sheep fed once a day (12, 13).

The great difference in counts in the two experiments when the animal was fed grain can not be explained on the basis of present knowledge. The pH values of rumen contents during the two experiments seemed to correlate with the difference in counts. The pH values in the first experiment were 7.2, 7.4, 6.5,

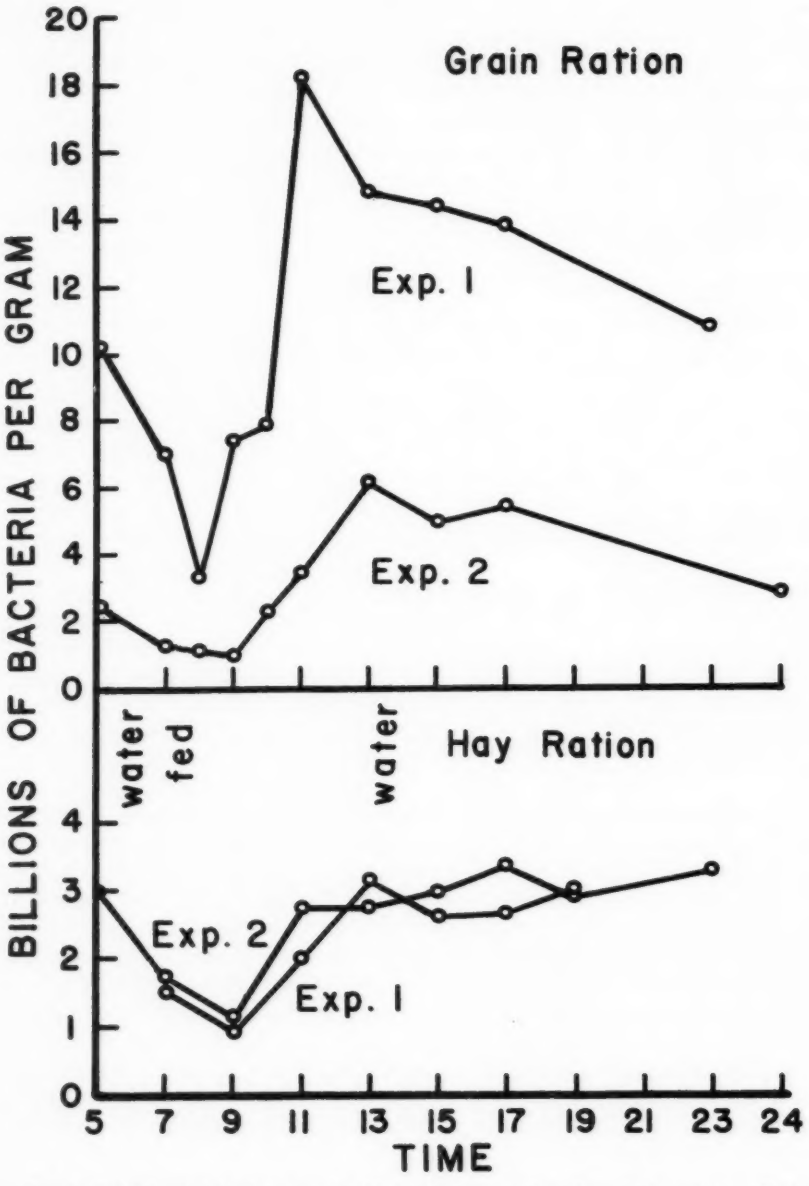

FIG. 1. Total bacterial colony counts obtained from samples of ruminal contents collected at various times of the day from a cow fed alfalfa hay or grain once a day.

6.0, 5.5, 5.9, 5.9, 6.3, 6.5, and 7.2; and 7.2, 7.2, 6.5, 6.2, 6.1, 6.0, 5.7, 5.9, 6.3, and 6.7 in the second experiment for the 5:00 to 23:00 samples, respectively. Where the bacterial count increased faster and reached a higher value, the pH decreased and then returned to the prefeeding level faster. It would be of interest to attempt to correlate these differences in rumen bacterial counts on high grain rations with other factors, such as the length of time the animal is on the ration, numbers of protozoa, species of bacteria present, etc. A similar large and significant difference was obtained between two animals sampled once a day (6 hr. after feeding) and fed mainly grain, and the difference correlated well with a lower pH and lack of protozoa in the animal with the higher bacterial count and significant differences in the kinds of predominant bacteria isolated (5).

Contrary to the conclusions of Wilson and Briggs (15), the present study shows that viable counts of ruminal bacteria do vary significantly, depending on the time of sampling and ration fed and, possibly, other factors. It seems possible that information on the trend in numbers of bacteria in the rumen through the day or on rates of growth obtained by using the present cultural procedure in conjunction with in vitro incubations of rumen contents, such as those used by Carroll and Hungate (6) to determine rates of volatile fatty acid production, may yield valuable information on the effect of various factors on the ruminal fermentation which would not be evident by determination of numbers of bacteria at one time during the day.

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FORAGE NUTRITIVE VALUE AS AFFECTED BY FERTILIZER APPLICATION

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SUMMARY

The digestibility of orchardgrass was determined as affected by fertilizer application. Results showed that under favorable conditions, digestibility, as well as dry matter and energy yields of forage, can be significantly increased by the use of fertilizer. Under unfavorable conditions, few or negative digestibility effects were noted.

Increasing plant pigment content was found to be associated with higher forage digestibilities. Under favorable conditions, fertilizer treatment raised the pigment content of forage.

Under favorable growth conditions, aftermath forage harvested on the same date but given dissimilar fertilizer treatment was found to differ in digestibility and feeding value.

Digestibility data showed divergence among the digestibility methods used. Not only did each of the three pigment methods differ significantly from the standard total collection method but they also differed significantly from each other.

The variable response of forage crops to fertilizer applications has been studied extensively. Ward (10) has recently and adequately reviewed this subject. He concluded that the divergent results frequently ascribed to fertilization were due to several factors, such as soil types, relative level of soil fertility, ratios of available nutrients, plant species, and climatic conditions such as temperature, precipitation, humidity, and wind velocity. In addition, Markley *et al.* (4), using 200 lb. of nitrogen per acre, reported a significant increase in the apparent digestion coefficients of protein and TDN of bromegrass and orchardgrass rations over those of low-N rations.

The objectives of this experiment were to investigate the response of forage digestibility to fertilizer treatment, and to study the effect of fertilizer on plant pigment concentration and its relationship to forage digestibility when determined by both standard and pigment methods.

EXPERIMENTAL PROCEDURE

Trial 1. After the first cutting had been removed, a section of an orchardgrass field was treated with 100 lb. ammonium nitrate per acre. No fertilizer was applied to the remaining portion of the field. During early growth the fertilized grass was taller and greener than the unfertilized forage. However, a severe drought (0.29 in. of rainfall in 17 days, accompanied by a hot, dry wind) occurred, which caused the color difference between the grasses to disappear, and the plants to become dry and withered. At the time of the digestion trial the entire field had a greenish-brown color.

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Six cows in milk were divided randomly into two groups of three for the digestibility determination of the two forages. Ration fed was the fresh-cut grass, except for daily supplements of 100 g. bonemeal and 50 g. salt. The herbage was cut daily in the early afternoon. One-half of the ration was fed immediately after cutting; the other half was fed the following morning after overnight storage at 0° C. Digestion trials were four days in length, preceded by a ten-day preliminary period. A two-day lag was allowed for the passage of food through the animals. Duplicate samples of the grasses, feces, and refused feeds were taken daily and composited for the period. Chemical analyses of these samples were made according to methods specified by A.O.A.C. (1). Dry matter digestibility was determined by four methods: total collection, plant pigment ratios (chromogen) (8), fecal pigment formula (7), and pheophytin ratios (3).

In Trial 1, it was difficult to extract the plant pigment completely, because the grasses resembled hay more than freshly cut grass. Apparently, the drought was severe enough to partially cure the grass while standing in the field. An indication of the severity of water deprivation in the trials is shown by the average dry matter of the pasture grasses as harvested: Trial 1, 41.2%, Trial 2, 28.9%, Trial 3, 26.2%.

Trial 2. The experiment was repeated the following spring, after the first cutting had been removed. It should be noted that after Trial 1 had been completed, 100 lb. of ammonium nitrate was applied to the unfertilized area to equalize as nearly as possible the fertility of the two areas. In the second trial, 500 lb. of a 5-10-5 commercial fertilizer was applied per acre to the same section of the field. With no apparent moisture limitation, the effect of the fertilizer could be observed throughout the growth of the plants. At the time of the digestion trials, the fertilized grass was definitely greener in color and the growth was taller and thicker than the unfertilized forage. The appearance of the unfertilized grass as fed in Trial 2 was noticeably coarser and rougher than the fertilized forage. The feces in Trial 2 also differed, in that the feces from the fertilized grass appeared to be of a finer texture, wetter, and darker than the other. Fertilization and no apparent moisture limitation apparently caused greater vegetative growth in this trial. Digestion trials were conducted in the same manner as described in Trial 1.

Trial 3. In this trial a different field of orchardgrass was used. A 5-10-5 commercial fertilizer was spread on a center section at the rate of 500 lb. per acre. Unfortunately, a heavy rainstorm in which 9.38 in. of rain fell in 24 hr., occurred two days after the fertilizer application. Since the field had a fair amount of slope, and since no evidence of greener color, or greater plant growth, was observed between the treated and untreated forage for 25 days, it was assumed that the applied fertilizer had been washed or leached away by the heavy storm. Ammonium nitrate, 100 lb. to the acre, was then distributed over the fertilized area, and a clear difference in color and growth was observed in a short time. When the grass was in pasture stage, digestion trials were performed as in trials above. Grasses in Trial 3 were held back somewhat by dry weather in September.

The two fields used had been in a fertilization program designed to maintain adequate levels of soil phosphorus and potassium to support a combined grass-legume sod. In spite of the program, the legumes had largely disappeared at experiment time. As an example of fertilization applied to fields, the Trial 3 field had received per acre: in the year 2 yr. previously, 12 tons cow manure, 500 lb. 0-10-20, and 1 ton lime; year previously, 500 lb. 0-10-20; experiment year, 800 lb. 0-10-20, in addition to fertilizer reported in this paper.

In view of the previous fertilizer treatments, soil phosphorus and potassium were believed to be adequate before the trials started, and no response to the new phosphorus and potassium added in Trials 2 and 3 was likely to occur. Responses obtained, therefore, are considered essentially due to nitrogen and this study may be regarded as basically a nitrogen study.

RESULTS AND DISCUSSION

Forage composition. The chemical analyses of the forages fed in the trials are shown in Table 1. Apparently, fertilizer application increased the protein content of the fertilized herbage in Trials 2 and 3, by 35% and 17% respectively, but not under the conditions of Trial 1, where drought and possibly

TABLE 1

Chemical analysis of orchardgrasses (dry matter basis)

Trial	Treatment	C.P.[a]	C.F.	E.E.	Ash	N-F.E.
1	Fertilized	15.3	28.4	3.79	6.25	46.2
1	Control	15.3	26.6	4.34	7.64	45.2
2	Fertilized	18.1	27.3	3.72	7.82	43.1
2	Control	13.4	32.1	3.00	7.64	44.9
3	Fertilized	16.4	28.2	3.94	10.7	40.7
3	Control	14.0	28.2	3.82	11.8	42.2

[a] C.P. = crude protein; C.F. = crude fiber; E.E. = ether extract; N-F.E. = nitrogen-free extract.

differences in the plant growth cycle interfered. Other components of the grasses were without significant differences, except for the high crude fiber and low ether extract of the unfertilized grass of Trial 2.

Digestibilities. The average component digestibilities of the grasses are reported in Table 2, with their TDN and CNE values. Dry matter, crude fiber, and nitrogen-free extract digestion coefficients of the fertilized orchardgrass were significantly lower in Trial 1, significantly higher in Trial 2, and without significance in Trial 3. The protein digestibility of the fertilized grass was found to be significantly higher in Trials 2 and 3, in accord with their increased protein content. In Trial 1, the protein digestibility of the fertilized grass was significantly lower than that of the unfertilized grass. Only in Trial 2 did the fertilizer application significantly improve the nutrient value of the orchardgrass. Increases of 9% in TDN and 17% CNE were found for the fertilized forage in this period.

TABLE 2

Average component digestibility coefficients of orchardgrasses and their TDN [a] and CNE [b] values

Period	Treatment	DM [c]	C.P.	C.F.	N-F.E.	E.E.	Ash	TDN	CNE	PP [d]
I	Fertilized	58.1	61.3	65.2	60.8	−4.1	35.6	56.0	43.7	382
I	Control	61.7	64.0	69.7	65.4	−5.4	44.6	57.9	46.0	396
II	Fertilized	64.2	71.0	67.6	66.4	21.5	44.8	61.8	51.4	626
II	Control	57.2	63.7	62.2	59.5	13.1	34.5	56.3	43.8	389
III	Fertilized	61.0	63.7	73.0	64.4	23.0	26.9	59.4	48.1	623
III	Control	60.3	58.6	73.7	66.4	18.9	22.2	58.6	46.7	527

[a] TDN = Total digestible nutrients, Morrison (6).
[b] CNE = Net energy by regression formula of Moore *et al.* (5).
[c] DM = Dry matter.
[d] PP = Plant pigment.

Length of growing time. While it is known that the effect of growing time has a significant effect on the digestibility of first-cut forages, its influence on second- and third-growth forages is not clear. No first-growth forage was used in the trials. Although all forages were harvested in the pasture stage, differences in growing time were noted between trials. In Trial 1, fertilizer was spread August 3, and the feeding trials started September 26, 54 days later. The corresponding plant growth time in Trial 2 was much shorter, 23 days, May 6 to May 29. In Trial 3, the first fertilizer application was made August 10, and the second on September 6. Digestion trials began on October 2, 26 days after the second fertilizer, or 53 days after the first fertilizer treatment. It is of interest that the fertilized grass with the shortest growing time had the highest digestibility, the greatest net energy, and was the only grass to show a significant digestibility increase for the applied fertilizer. Supposedly, this effect might be due to greater growth stimulus present in the orchardgrass in May compared to September. However, other factors could also be involved.

In Trial 1, it is true that under normal conditions the amount of fertilizer applied could have been consumed well within the 54 days' growing period. However, it is felt that the main effect was due to drought, and it would be difficult to attach significance to the length of the growing period in Trial 1, when water deprivation apparently had stopped growth and the forage plants stood inactive in the field for almost 2 wk.

It appears that fertilization can affect the composition and digestibility of aftermath orchardgrass. Digestibility data show that second-cutting forage harvested on the same days, but receiving different fertilizer treatment, can vary considerably in component digestibilities as well as in TDN and CNE values.

Forage yields. Results of the effect of fertilizer on forage yield are shown in Table 3. Measurements on area and yields were not taken in Trial 1.

In this experiment, fertilizers appear to have a greater effect on yield than on digestibility. However, digestibility increases are desirable, since they tend to give higher net energy values per acre, as shown in Table 3. For example, in Trial 2 the added fertilizer produced an increase of 38% in CNE, due to gains of 17% in yield and 12% in digestibility. This surpassed the 29% CNE

TABLE 3

Fertilizer effect on forage yields

Trial	Treatment	D.M. lb/acre	% Gain	TDN/ acre	% Gain	NE/acre	% Gain
1	Fertilized	No data taken	....	....	....	....	....
1	Unfertilized	No data taken	....	....	....	....	....
2	Fertilized	1,369	17	845	28	705	38
2	Unfertilized	1,175		659		511	
3	Fertilized	1,776	25	1,018	31	854	29
3	Unfertilized	1,419		780		663	

increase of Trial 3, which was based on a higher gain of 25% in yield and a lower gain of 1% in digestibility.

Plant pigment. Definite color differences could be seen between the fertilized and control grasses at the time of cutting, except in the case of Trial 1. This visual color appraisal was substantiated by spectrophotometric data, shown in Table 4, when the plant pigment content of the forages are reported in A-values

TABLE 4

Plant pigment content of orchardgrasses fed, read at two different wave lengths

Trial	Fertilized	Unfertilized	% Gain	Fertilized	Unfertilized	% Gain
	(406 mμ)			*(415 mμ)*		
1	382	396	− 4	519	515	+ 1
2	626	389	+61	939	563	+67
3	623	527	+18	929	771	+20

per gram of dry weight. Under the conditions of Trial 1, there were no pigment differences. However, increases of 64 and 19% in pigment content were produced by the fertilizer in Trials 2 and 3, respectively.

The average dry matter digestibilities of the forages, calculated by four methods, are reported in Table 5, along with the gain or loss due to fertilizer treatment. It is apparent that the fertilizer effect on digestibility was different in each trial. Statistical analysis (9) of the combined trials showed a highly significant ($P < 1\%$) interaction of trials by treatment, which will be described later.

Since cow and error terms of the trials were found not to be uniform, an analysis of variance was conducted on each trial separately and reported in Table 6. The effect of fertilizer treatment on digestibility as shown by four methods agreed with total collection results, in finding that the treated forages had digestibilities that were: significantly lower in Trial 1, significantly higher in Trial 2, and without significance in Trial 3.

Cow differences were found to be highly significant in Trials 2 and 3. Cow significance in Trial 2 was due apparently to one animal in the unfertilized

TABLE 5

Dry matter digestibilities by different methods and differences due to fertilizer

Trial	Method	Unfertilized	Fertilized	Difference
1	T.C.*	61.7	58.1	−3.6
1	P.P.	70.1	66.4	−3.7
1	F.P.	61.8	59.6	−2.2
1	Ph.	68.4	63.1	−5.3
2	T.C.	57.2	64.2	+7.0
2	P.P.	67.6	70.8	3.2
2	F.P.	59.5	65.9	6.4
2	Ph.	64.4	66.1	1.7
3	T.C.	60.3	61.0	0.7
3	P.P.	70.9	72.5	1.6
3	F.P.	63.9	66.7	2.8
3	Ph.	67.3	68.1	0.8

* T.C. = Total collection; P.P. = plant pigment; F.P. = fecal pigment formula; Ph. = pheophytin.

TABLE 6

Analyses of variance of dry matter digestibilities

Source of variation	Degrees of freedom	Error term	Mean squares		
			Trial 1	Trial 2	Trial 3
Treatment (T)	1	C	81.40**	125.12**	12.91
Cows (C: T)	4	R	0.67	5.41**	19.20**
Methods (M)	3	R	96.39**	80.22**	127.83**
T × M	3	R	2.51*	9.53**	1.27
MC: T (R)	12		0.64	0.86	3.00

* Significant at 5% level.
** Significant at 1% level.

group that consistently maintained low digestibilities. Cow digestibilities in Trial 3 were scattered.

The highly significant differences found for methods indicated a lack of concurrence between the digestibility methods used. Duncan's (2) multiple range test (Table 7) at the 5% level showed that the digestibility coefficients of three pigment methods tested not only were significantly different from the standard total collection data but also from each other. In 18 comparisons, fecal pigment method averaged 4% higher in digestibility than total collection, while pheophytin and plant pigment were 10 and 15% higher, respectively.

The interaction between treatment and method was found to be significant in the first two trials. However, the test for significance in both was very sensitive, due to the low error term involved.

With trials combined, a highly significant interaction between treatment and trials was observed. This indicated that the effect of fertilizer was dependent on the trials or on the climatic conditions that existed at the time of the trials. The dominant climatic feature of Trial 1 was the extremely dry weather, with the resulting lack of water available to the forages for plant metabolism and growth. This lack of water may also have been true in Trial 3,

to a lesser extent. The average daily rainfall for growing time of Trials 1 and 3 was less than 0.04 in.; for Trial 2, 0.08 in. Results, therefore, are interpreted to indicate in Trials 1 and 3 that the fertilizer effect was limited by water deprivation. The facts that Trial 2 was conducted in the spring rather than in the fall, and that the more complete fertilizer was used in Trial 2, may have been contributing factors to the increased efficiency of the fertilizer effect on digestibility observed in this period.

TABLE 7

Average method digestibilities

Methods	Digestibilities	Statistical significance
T.C.	60.4	[a]
F.P.	62.9	|
Ph.	66.2	|
P.P.	69.7	|

[a] Statistically different at 5% level.

The use of fertilizer increased the plant pigment content of the treated over the control forages by 1, 67, and 20%, respectively, in the three trials. The corresponding dry matter digestibility increases for fertilizer treatment were −6, 12, and 1%. The regression of the total collection digestibility on plant pigment was found to be positive in slope and significant at the 5% level. The regression equation expressing this relationship was: $Y = 54.6 + 0.0082\ X$ where Y = dry matter digestibility and X = A values per gram of dry forage at 415 mμ. If the data of Trial 1 are excluded, due to the severe, dry weather and the negative digestibility effect obtained for the applied fertilizer, regression becomes $Y = 49.1 + 0.0145\ X$. The standard deviations from regression were 2.68 and 2.70, respectively, for the above equations. Correlation coefficients for the relationship, 0.51 and 0.67 respectively, were significant at the 5% level. Fragmentary data above indicate a gain of 1.5 digestibility units for every increase of 100 units of plant pigment in pasture orchardgrass.

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EFFECT OF FEEDING PELLETED COMPLETE RATIONS TO LACTATING COWS

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SUMMARY

Pelleted complete rations were compared to a conventional ration when fed to lactating Milking Shorthorn cows in a short-term reversal study and a longer-term, continuous study. No digestive disturbances or depression in the milk fat percentage were observed. The results are discussed briefly.

Abnormally low milk fat tests have been reported when pelleted rations have been fed to dairy cows (1, 3, 4, 8, 9). Similar but less consistent results have been reported when ground and pelleted hay has been fed (5, 7, 10). It has been suggested that factors other than the type and physical nature of the hay are involved. Some of the factors mentioned were type and proportion of concentrates in the ration, total amount of hay offered, and the breed of the animals being fed (2, 4).

The present report concerns observations on the milk fat test of lactating Milking Shorthorn cows, when a ration fed in the conventional manner was compared to rations fed in a ground, mixed, and pelleted form.

EXPERIMENTAL PROCEDURE

In the fall of 1959, a short-term study was conducted in which a control ration of baled alfalfa and grass hay, plus a dairy concentrate fed as a meal, was compared to a pelleted low-concentrate ration, a pelleted high-concentrate ration, and a pelleted high-concentrate ration plus low-quality grass hay scattered on the floor of the pen. The hay was added in an effort to prevent the anticipated fat depression accompanying the feeding of pelleted complete rations. The animals were kept in individual pens bedded with sawdust. The dairy concentrate was 30% corn, 20% oats, 20% wheat bran, 20% corn gluten, 5% linseed oil meal, and 5% soybean oil meal. The rations are illustrated in Table 1. No binders were used in the pelleted rations. The amounts of feed offered were such that each group would consume approximately the same amounts of TDN per day, except for the added hay consumed by animals on Ration III. Adjustment periods and experimental periods were seven days in length. Weekly milk fat values were determined on milk samples composited from four consecutive milkings. The four mature animals, all of the Milking Shorthorn breed and in late lactation, were milked twice daily. Feed consumption and milk production values were recorded daily. The animals were weighed weekly. The experimental design was that of a single, standard 4×4 Latin-square not balanced for carry-

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TABLE 1

The experimental rations

Control	I[a]	II[b]	III
	Low-concentrate pellet	High-concentrate pellet	High-concentrate pellet
Dairy concentrate (up to 8 lb/day)	20% Dairy concentrate	2.5% SBOM 10% molasses 62.5% corn and cob meal	
plus		25% timothy hay	
No. 2 alfalfa hay	40% No. 2 alfalfa hay		plus 10 lb. Grass hay
Grass hay	40% Grass hay		

[a] Ground through a 3/8-in. screen and extruded from a 3/8-in. die.
[b] Ground through a 3/8-in. screen and extruded from a 5/8-in. die.

over effects. The purpose of the study was to determine if satisfactory levels of feed intake could be obtained with an absence of pathological response.

In the spring of 1960, after the results from the reversal study were summarized, a group of 16 Milking Shorthorns were allocated to two experimental groups on the basis of age and first-lactation milk and fat production. The animals were put on experiment two days after calving, to lengthen the period that the animals would be consuming a pelleted ration, and because it appeared to be the time when one would be expected to start feeding such a ration. Prior to freshening, all animals had been receiving hay and corn silage with no concentrates.

The control animals were offered all the alfalfa hay they would eat at the morning feeding and all the grass hay they would consume at the afternoon feeding. The concentrate fed was increased to 8 lb. per day within the first 10–14 days and held at that level for the first 70 days of the trial. After 70 days on experiment, the daily concentrate allowance was cut 0.5 lb. each succeeding week. The dairy concentrate was the same as used in the reversal study.

The animals allocated to the pelleted ration treatment were switched to the pellets (Ration I, Table 1) two days after freshening, when they entered the milking string. They received nothing but pellets from then on. The components of the pelleted ration were from the same source as those of the control ration. The amounts of pellets offered were such that the cows would just barely consume them between feedings. This procedure was not altered as the lactation progressed. The animals were bedded with sawdust in a standard stanchion-type barn and allowed to exercise in a dry lot when not feeding.

The procedures for keeping milk and feed records and milk fat determinations were the same as in the reversal study. The animals were to remain on their respective treatments for the entire experimental period, which was tentatively set as 100 days. Body weights were recorded weekly.

RESULTS AND DISCUSSION

Treatment averages for the reversal study are presented in Table 2. Variations in milk fat percentage or production were not statistically significant.

TABLE 2

Response of cows fed pelleted complete rations in a reversal-type study

Av. daily performance per cow	Ration				Standard error of mean
	Control	I	II	III	
4% FCM *(lb.)*	14.9	14.7	16.5	14.4	±0.85
Milk fat *(%)*	3.6	3.4	3.5	3.2	±0.20
Dry matter intake *(lb.)*	35.4	32.6	30.0	37.5	±0.91
Calculated TDN intake *(lb.)*	19.1	17.8	19.5	23.4	±0.40
Lb. FCM/lb TDN	0.78	0.82	0.85	0.62	±0.05

Prior to the experiment, the average milk fat test was 3.5%. Milk production values also were similar. Average dry matter intakes observed for the pellet plus bedding hay were higher than for the other groups. Actually, a considerable amount of bedding hay was trampled in with the sawdust. No digestive disturbances were observed.

While the periods were short in this study (seven-day adjustment and seven-day experimental), Ronning (9) has observed milk fat depression to occur after only 1 wk. on a pelleted ration. Conversely, Hand (4) reported a 5-wk. period prior to milk fat depressions.

It was decided that either of the pelleted rations would be suitable for further use in studying the problem and that bedding with hay was not necessary. However, since none of the cows available was expected to produce more than 40 lb. of milk a day, the low-concentrate ration was considered the most appropriate. Preliminary feeding had indicated that the high-concentrate ration would be consumed in amounts far above those recommended for animals in their production class.

Due to low production and mastitis, three animals in the control group and two animals in the experimental group were removed from the experiment prior to the prescribed 100 days.

To utilize the available information in respect to a possible treatment effect on milk fat test, a least-squares analysis was conducted to test the difference between treatment regressions by the mean square for the differences among animal regressions within treatments. The analysis indicated that there was no significant difference between the regressions (per cent milk fat × weeks) of the two treatment groups. These results are in contrast to those of other workers (1, 3, 4, 8, 9), who observed a decrease in per cent of fat in milk from animals consuming complete pelleted rations. The contrasting results may be caused by the presence of a poor-quality grass hay in the ration and/or, as compared to the work of Ronning (9), the coarser grind and larger pellets used in this study. In view of Hand's (4) report, there might be a breed difference. However, it should

be noted that the animals in this study were losing weight as the experiment progressed. Conversely, apparent gains in body weights in conjunction with low-fat tests were reported by Hand (4), Ronning (9), and Fossland and Fitch (3). The hypothesis of McClymont and Kondos (6) suggests that milk fat depression would be less likely if there were no gains in body weight and depot fat was being mobilized. Van Soest (11) has observed, from a limited amount of data, that when animals consume a milk fat depressing ration in liberal amounts and then consume submaintenance levels, the milk fat test rises. The observations of the latter workers (6, 11) may be applicable to the results reported herein.

The average production and feed consumption values for the animals which completed the experiment are presented in Table 3.

TABLE 3

Response of cows fed a pelleted complete ration

Av. daily performance per cow	Control[a]	Experimental[b]
Milk *(lb.)*	32.4 ± 2.18[c]	29.3 ± 0.60
Milk fat *(%)*	3.96 ± 0.23	4.01 ± 0.17
Milk fat *(lb.)*	1.28 ± 0.15	1.18 ± 0.06
4% FCM *(lb.)*	32.1 ± 3.04	29.4 ± 1.06
Liveweight gain *(lb.)*	−0.59 ± 0.60	−0.35 ± 0.35
DM intake *(lb.)*	32.9 ± 0.17	30.4 ± 1.24
Calculated TDN intake *(lb.)*	18.2 ± 0.04	16.7 ± 0.67
Concentrate intake *(lb.)*	7.3 ± 0.04	6.1 ± 0.24
Alfalfa intake *(lb.)*	13.6 ± 0.10	12.2 ± 0.15
Grass intake *(lb.)*	12.0 ± 0.07	12.2 ± 0.15
Lb. FCM/lb TDN	1.76 ± 0.17	1.76 ± 0.11

[a] There were five animals in the control group and six animals in the pellet group. The experimental period was 98 days in length.

[b] ⅜-in. pellet; the hay was ground through a ⅜-in. screen.

[c] Standard error of the mean.

The regression coefficients of milk fat per cent by weeks for the within cow control and pellet animals were −0.050 and −0.069, respectively. The average body weight for the control and experimental animals was 1,292 and 1,262 lb. The average milk fat percentages for the two groups were similar. The average daily 4% FCM production appeared greater for the controls but, since they consumed more dry matter and TDN, the average pounds of 4% FCM produced per pound of TDN consumed were similar. The average weekly milk fat percentages are illustrated in Figure 1. It is apparent that no precipitous drop in milk fat percentage occurred in either group.

The results of this experiment suggest that pelleted complete rations can be fed to lactating cows without depressing the milk fat test. Whether pelleted rations containing higher proportions of available energy can be successfully fed to high-producing cows remains to be tested. It also appears possible that rations could be devised for various production levels, since the experimental animals in this study were essentially at full feed and still were not consuming more than the recommended amounts of energy. Such a procedure could be advantageous to a highly mechanized operation.

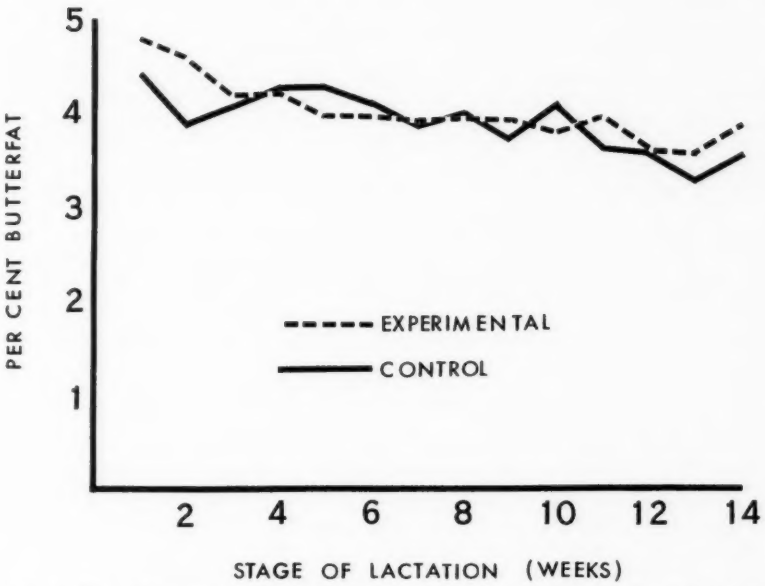

FIG. 1. Average percentages of milk fat by weeks for cows consuming a complete pelleted ration and the same ration in conventional form.

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A STUDY OF FACTORS AFFECTING RATE OF INTAKE OF HEIFERS FED SILAGE

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SUMMARY

Dairy heifers were used to study factors that influence the rate of consumption of alfalfa silage. Consumption was linearly and positively related to dry matter content of the silage. However, this was shown to be a secondary relationship, since changing the dry matter content of silage or hay at time of feeding did not alter rate of consumption. It was concluded that the dry matter content of he forage when ensiled, and the resulting fermentation process, are very important factors in determining the rate of consumption of the resulting silage.

In trials of a preliminary nature, adding limestone or other minerals, ammonium salts, molasses, commercial flavor preparations, concentrates, potassium orotate plus methionine, and an aureomycin-containing feed additive to silage had no effect on rate of silage consumption, whereas glucosamine, large amounts of lactic plus other silage acids, thyroprotein, and thiouracil had a depressing effect. The depressing effect of silage on intake is probably a metabolic effect on appetite and not a direct effect through palatability.

Intake of silage and hay was reduced when the effluent liquid from a silo, aqueous extract of silage, residue after ether extraction of the effluent liquid, large quantities of lactic acid or lactic plus other silage acids, a mineral mixture, glucosamine, urea, or other ammonium salts were placed directly into the rumen. Similarly, hydrolyzed casein, tyramine, sucrose, water, acidic or basic ether soluble fractions of the silo effluent, or low levels of the silage acids had no such effect on intake. The desirability of further investigations is indicated.

It has been shown that heifers fed most silages voluntarily restrict their rate of intake below that of contemporary heifers fed hay (4, 5, 6, 7). Similar, though smaller, differences between silages have also been observed (1, 4, 6). It has been suggested that differences in dry matter content and/or other chemical constituents of the silages may be factors in determining the rate of voluntary consumption of that silage. Attempts to delineate such factors that might influence intake have been performed and the present report deals with a number of diverse trials to determine whether dry matter consumption from silage could be altered by adding various materials to the feed or directly into the rumen, and to ascertain the effect of giving certain silage constituents when hay was fed.

EXPERIMENTAL PROCEDURE

All these trials were conducted using growing dairy heifers. All heifers were 8 to 20 mo. of age when the trial started, except those in Trial A-2, which were 5 mo. of age. During the several trials, alfalfa silage or hay was the only feed, except for calves under 8 mo. of age, which were fed 2 lb. of grain daily. The moisture content of all feeds was determined during each trial.

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These trials, designed to study the effect of various factors on consumption, have been divided into four main experimental groupings, namely: Group A Experiments—The effect of water content of ration on consumption; Group B—The effect of silage constituents on hay consumption; Group C—Effect of silage constituents on silage consumption; Group D—Effect of various supplements added to silage on silage consumption.

Further procedural details will be given with each trial. In studies involving the addition of materials directly to the rumen, and estimating their effect on intake, the material under investigation was allowed to drip into the rumen in four equally divided doses at 4 A.M., 7 A.M., 12:30 P.M., and 4:15 P.M. It required 5 to 25 min. for complete intraruminal administration. A small stainless steel or rubber rumen fistula was used (2, 3).

RESULTS

Group A Experiments—Effect of water content of ration: (A-1 effect of moisture content of silage on dry matter intake.) Several silages containing from 18 to 54% dry matter (D.M.) were fed ad libitum to 14 heifers. The silages were fed in sequence for ten to 15 days each, with each heifer receiving three to nine different silages. The sequence varied according to the availability of silages and heifers. A positive correlation ($+.79$; $p = < 0.01$) was obtained between daily D.M. intake per 100 lb. body weight and D.M. per cent of the silage. The regression equation was $\hat{Y} = 0.694 + 0.039X$, where $\hat{Y}$ = D.M. intake per hundredweight and X = D.M. per cent of the alfalfa silage. The deviation from linearity was not significant and $Sb = 0.00008$. The relationship between D.M. content of silage and pounds consumed per day was also linear and positive ($r = .71$, $p = < 0.01$). The regression equation was $\hat{Y} = 2.12 + 0.22X$. These regressions are shown in Figure 1. A similar relationship between voluntary intake of silage and D.M. content has been found with cows (1).

(A-2 effect of dried silage on intake and gain.) A preliminary trial in which silage was dried daily on a forced warm air (80° C.) dryer and then fed to three heifers for a few days indicated that there was no change in D.M. consumption.

To further study the effect of moisture content of silage on intake, a large amount of wilted alfalfa silage was dried to 90% D.M. on a mow dryer, using heat, and then stored before being fed to five calves for a 7-mo. period. The gain and intake of these heifers were compared to heifers fed hay and the regular wilted alfalfa silage from the same source. Detailed information on this experiment was reported previously (6). The comparative gains and intakes are presented in Table 1. The voluntary intake and gain in body weight of heifers fed dried silage were no different from those of heifers fed the same wilted silage with its usual moisture content.

(A-3 effect of adding water to hay.) Two heifers were fed a sequence of long alfalfa hay, then chopped hay soaked in water for 6 hr., and back to long alfalfa hay. This sequence was repeated three times with each calf and each treatment was of ten days' duration. Dry matter determinations were per-

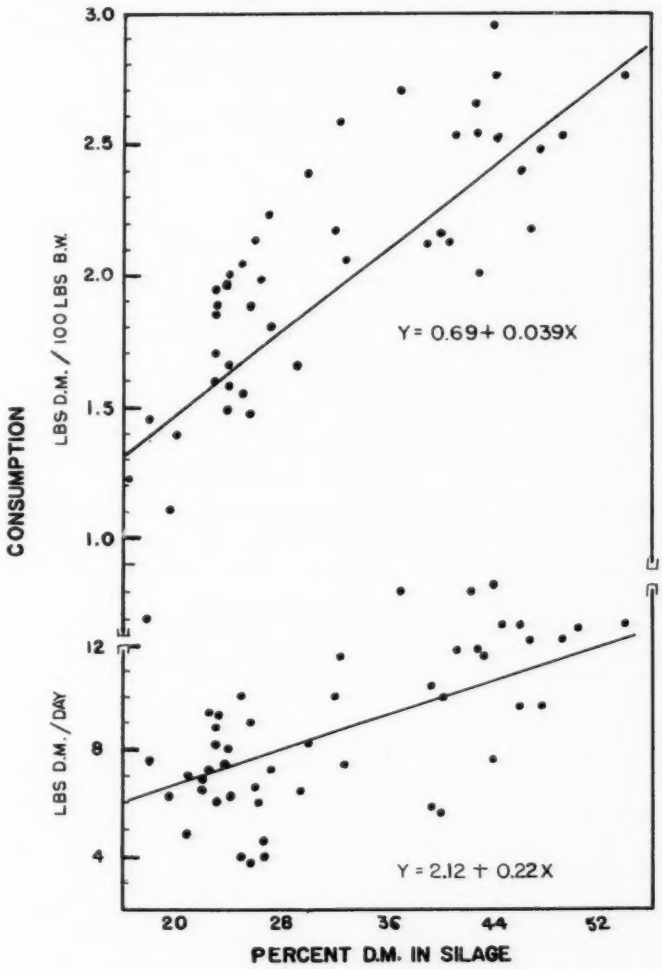

FIG. 1. The relation between voluntary consumption of alfalfa silages and their dry matter content, using dairy heifers.

TABLE 1

Comparison of gain and feed consumption of dairy heifers fed alfalfa hay, wilted alfalfa silage, and dried alfalfa silage from 5 to 12 mo. of age [b]

Type of forage fed	Number of calves	Body weight gain [a]	Dry matter consumed [a]	Dry matter intake per 100 lb. body weight [a]
		(lb/calf/day)	*(lb/calf/day)*	
		Holstein calves		
Hay	9	1.54 ± .19 [A]	11.64 ± .85 [A]	2.66 ± .19 [A]
Silage	9	.92 ± .23 [B]	8.65 ± 1.05 [B]	2.32 ± .12 [B]
Dried Silage	3	.91 ± .13 [B]	8.19 ± 1.12 [B]	2.33 ± .04 [B]
		Jersey calves		
Hay	11	1.01 ± .32 [A]	7.87 ± .96 [A]	2.77 ± .24 [A]
Silage	11	.40 ± .15 [B]	5.01 ± .73 [B]	2.33 ± .20 [B]
Dried Silage	2	.57 ± .19 [B]	5.53 ± .95 [B]	2.28 ± .03 [B]

[a] Figures given are averages ± standard deviation; those averages with the same letter as superscript form a statistically homogeneous group, $P < 0.01$.

[b] Molasses added to dry silage of all five animals during the last month—no improvement in intake.

formed on the hay and material refused. The average consumption when fed wet hay was 1.5 lb. D.M. more than when long dry hay was fed. This treatment sequence was repeated with a third calf and the consumption of this calf decreased several pounds every time that soaked hay was fed.

Two other calves were fed soaked hay for a 95-day period. Their consumption was compared with contemporary calves receiving the same hay and is presented in Table 2. The soaking of the hay with water did not noticeably change the amount of dry matter consumed by these heifers. With most heifers, the soaking of hay with water did not alter the amount of D.M. consumed.

TABLE 2

Hay dry matter consumption as influenced by soaking with water

Group	No. heifers	Daily D.M. intake		
		Before soaking	During soaking	After soaking
		(lb/heifer)		
Experimental	2	6.8	8.9	11.5
Control	12	7.2	8.9	11.0
Day in period		30	95	30

(A-4 effect of adding water to silage.) In five trials water was added to silage 4 hr. before feeding, to study the effect of reducing the D.M. content of the forage consumed. Consumption was compared to the amount consumed when the silage was fed with its normal moisture content. In four trials, the experimental periods were of ten-day duration, but in one trial they were of 30 days' duration. The sequences used, the D.M. content of the roughages fed, and D.M. consumed are presented in Table 3. Trials A through D indicate that

TABLE 3

Dry matter consumed as affected by type of silage fed and by changing its dry matter content by adding water at feeding time (Experiment A-4)

	Period (10 days)							
	1	2	3	4	5	6	7	8
Trial A								
Forage sequence	D.C.[a]	Haylage [b]	Haylage	Haylage	.....	.....	.....	Hay
Forage D.M. *(%)*	20.9	43.3	22.2	46.0				88.0
Water added	.....	no	yes	no				.....
D.M. consumed *(lb.)*	7.03	11.76	13.05	12.90				13.4
Trial B								
Forage sequence	D.C.	Haylage	Haylage	Haylage	D.C.	.....	.....	Hay
Forage D.M. *(%)*	22.0	22.4	42.7	22.0	22.3			89.0
Water added	.....	yes	no	yes	no			.....
D.M. consumed *(lb.)*	6.70	13.00	13.19	14.50	9.30			14.50
Trial C								
Forage sequence	Haylage	D.C.	Haylage	D.C.	Haylage	Haylage	D.C.	Hay
Forage D.M. *(%)*	44.0	22.3	21.8	23.6	50.5	23.9	20.7	89.0
Water added	.....	no	yes	no	no	yes	no	.....
D.M. consumed *(lb.)*	14.2	7.2	11.0	7.6	12.5	12.7	4.9	12.7
Trial D								
Forage sequence	Haylage	Haylage	D.C.	Haylage	Haylage	.....	.....	Hay
Forage D.M. *(%)*	42.8	23.0	19.7	24.8	46.8			89.0
Water added	.....	yes	no	yes	no			.....
D.M. consumed *(lb.)*	11.58	12.72	6.16	13.51	12.22			14.2
Trial E								
Forage sequence	Wilted	Wilted	Wilted	Wilted	Wilted	Wilted	D.C.	.....
Forage D.M. *(%)*	27.2	30.2	30.2	24.4	32.6	30.6	23.0	.....
Water added	no	no	no	yes	no	no	no	.....
D.M. consumed *(lb.)*	6.4	7.4	8.2	9.2	9.5	8.0	7.5	

[a] Direct-cut alfalfa silage—no preservative.

[b] Alfalfa wilted to 40-50% before ensiling each value in Trials A, B, D, and E are the average of two heifers; Trial C = three heifers. Period 1 represents the last ten-day period of a 3-mo. feeding trial on that forage, except that in Trial E, where every period was 30 days in length.

water added to heavily wilted silage (haylage) did not alter its D.M. consumption by heifers when fed in a variety of sequences. In Trial E, water was added to wilted silage during a 30-day period with no change in the trends of dry matter consumption by the two heifers.

In Trials A through D the larger D.M. intake of haylage compared to the amount of direct-cut (D.C.) silage consumed was evident. These silages were harvested from the same field. There was little difference in the amount of D.M. consumed as haylage or alfalfa hay (Table 3). These results with short-term trials are in agreement with the results of longer-term trials (6).

The results from these trials indicate that the addition of water to bring the D.M. content of wilted silage from approximately 45 to 22% or from 30 to 24% did not result in any change in the D.M. consumption of the silage by heifers. Neither did the addition of water to hay affect consumption rate in the majority of the heifers.

(A-5 effect of adding water directly to the rumen.) Eight trials were performed in which 58 lb. of water were added to the rumen through a small fistula. Average daily hay consumption for the three days before and three days during intraruminal water administration averaged 11.5 and 11.5 lb., respectively. In other trials, hay consumption, during and after water administration, averaged 13.9 lb. and 13.6 lb/day. It is evident that addition of water directly to the rumen did not alter the amount of hay consumed.

The results of these trials suggest that dry matter content of the forage ensiled and resulting differences in fermentation appear to be major determinants for the amount of dry matter consumed in the form of silage rather than the dry matter content of the silage per se as it is consumed. This is true even though there is a general and positive relationship between the dry matter content of silages and their rate of consumption. This relationship is somewhat ficitious and probably more accurately relates intake to the fermentation process during the silage production and the "quality" of the resulting dry matter.

Group B Experiments—Effect of silage constituents on hay consumption: (B-1 intake of hay soaked with effluent from a silo.) Previous results (7) indicated a reduction in hay D.M. intake after the hay had been soaked with effluent from a silo. A subsequent trial with four heifers fed chopped hay soaked with effluent from a silo for 105 or 75 days showed a small comparative decrease in intake and body weight gain during this period. Three additional heifers were given chopped hay soaked with effluent from a silo for 50 to 70 days. There was a small decrease in intake. It decreased 1.5 lb/day/heifer when this treatment started and increased 3.8 lb/day/heifer when this treatment stopped. After ten days, they were again fed hay soaked with silo effluent for ten days and then back to regular hay. Intake decreased 2.9 lb/day/heifer when changed to hay soaked with silo effluent and increased 2.2 lb/day/heifer when changed to regular hay.

The results of these trials suggested that the inhibition of intake by effluent liquid from silos filled with alfalfa deserved further investigation.

(B-2 Effect on intake when silo effluent was given directly into the rumen.) In these trials, effluent from fully cured silos was put directly into the rumen through small fistulas. Consumption for three to seven days before and after was compared to the consumption during an experimental period of equal duration.

The results of 26 complete switch-over trials and eight one-way changes are given in the upper three lines of Table 4.

These results show that intraruminal administration of effluent liquid from a silo produced a decrease in voluntary intake of hay by heifers. Silo effluent was obtained from silos containing alfalfa, grass, and in one instance corn silage, during a 3-yr. period. The type of crop from which effluent was obtained did not appear to affect the response.

Hay intake was reduced the first day this material was given. Occasionally, intake would increase slightly as the trial progressed, but in most cases it remained at approximately the same level. Intake always increased the day after

TABLE 4

Intake of hay as influenced by intraruminal administration of effluent from silos and other materials (Experiments B-2 and B-3)

Material given	Amount	No. heifers	No. trials	Days in trial	Daily hay intake			
					Before	During	After	Change
					(lb/heifer)			*(lb/heifer)*
				Experiment B-2				
Silo effluent	48 lb.	8	26	3–7	14.4	9.8	14.4	− 4.6
Silo effluent	48 lb.	6	8	3	15.9	11.0	.....	− 4.9
Silo effluent	48 lb.	6	8	3	.....	11.3	15.3	− 4.0
Silo effluent	24 lb.	3	5	1	16.8	14.8	16.9	− 2.0
Silo effluent fractions:								
Acidic ether sl.	....	1	1	1	15.2	14.8	15.5	− .5
Basic ether sl.	....	1	1	1	14.7	15.5	13.1	+ 1.6
Ppt.	....	1	1	1	15.1	15.7	17.1	+ .4
Liquid residue	....	1	1	1	11.8	2.0	12.1	−10.0
Simulated alfalfa ash	1.8 lb.	2	2	1	17.7	16.2	17.6	− 1.4
Lactate	288 g.	3	3	3	15.2	14.9	14.8	− .1
Acetate	126 g.	3	3	3	14.6	15.4	16.0	+ .1
Propionate	100 g.	3	3	3	15.3	14.9	15.7	− .6
Mix three above	..... g.	3	3	3	14.8	13.6	14.8	− 1.2
Lactate plus acetate	576 & 252	2	2	1	14.0	11.1	14.2	− 3.0
Hydrolyzed casein	1.6 lb.	2	2	1–3	13.8	14.1	14.1	+ .1
Juice from green alfalfa	48 lb.	4	5	1–2	15.5	10.9	14.8	− 4.2
Glucosamine	1 lb.	2	4	3	18.5	18.4	19.4	− .6
Glucosamine plus NH_4Cl	1 lb.	1	1	3	20.4	17.6	20.7	− 3.0

intraruminal administration ceased, and only occasionally would it increase further on succeeding days.

This liquid contained 4 to 8% dry matter, of which over one-third was ash. Thus, approximately 2 to 4 lb. of D.M. as silo effluent containing only 1.3 to 2.6 lb. organic matter when put directly into the rumen caused a decrease in hay intake of 4 to 4.9 lb.

Fifty-two pounds of silo effluent was fractionated by extraction with ethyl ether while acidic and again while basic. After ether removal, the extracts were tested for effect on intake, using one heifer. A copious precipitate formed when the silo effluent was made basic and this material was filtered, resuspended, and tested. The final liquid was neutralized to pH 6.0 and tested. The results of these trials are also shown in Table 4—Lines 5 to 8. The residual liquid caused a very marked decrease in intake, whereas none of the other fractions affected intake. This fraction contained the original plus the added mineral matter.

A relatively large amount of a mineral mixture resembling the composition of alfalfa ash was given to two heifers. A small decrease (1.4 lb.) in intake occurred (Table 4—Line 9).

The reduction in intake that occurred when silages were fed and when silo effluent was put into the rumen may or may not be due to the same factor(s). Further work is needed to determine if the effect of these two materials has a common basis.

(B-3 Effect on hay intake by intraruminal administration of certain organic acids.) In four trials of ten to 30 days' duration, a mixture of 228 g. lactic acid plus 126 g. acetic acid plus 10 g. butyric acid neutralized to pH 5.0 was given intraruminally each day. No change in intake was noted.

In another trial of a Latin-square design, lactic acid—288 g., acetic acid—126 g., propionic acid—100 g., and all three acids combined were administered to three heifers. The acid mixture was neutralized to pH 5.0. The results of this trial are presented in Table 4, Lines 10 to 13. Hay intake decreased 1.2 lb/day/heifer when all three acids were given and less when only one acid was given. These changes were not statistically significant.

In one trial 576 g. lactic acid plus 252 g. acetic acid (neutralized to pH 5.0) were given to two heifers. Hay intake decreased 3.0 lb. in this trial (Table 4, Line 14). The amount of acids used in this latter trial is more than that found in 15 lb. of usual silage dry matter.

An HCl hydrolysate of casein (1.6 lb.-acidicase) was administered intraruminally to two heifers with no change in intake of hay (Table 4, Line 15).

Fresh alfalfa was macerated and the liquid expressed, using a cider press. This alfalfa juice amounted to 25% of the original weight of alfalfa and was tested for its effect on intake. It caused a marked depression in intake in four of the five trials performed (Table 4, Line 16). However, it did cause bloat in some cases and intake was still below normal for one or two days after treatment stopped. This alfalfa juice contained 13 to 15% dry matter, of which approximately one-eighth was ash. Thus, even though oral intake of hay was decreased when the juice was given intraruminally, total organic matter intake was increased by 2 to 3 lb/heifer/day. The effects of fresh alfalfa juice and silo effluent on intake were considered to be of a different nature.

Glucosamine has been shown to decrease the quantity of food consumed by rats (9) and this material was tested for its effect on heifers. When 1 lb. per day was given intraruminally in four doses no appreciable effect on intake of hay was noted (Table 4, Line 17). However, as mentioned later, when heifers were fed silage, it did cause a decrease in intake. Doubling the inorganic nitrogen given by adding an equal amount of nitrogen as NH_4Cl to the glucosamine did cause a decrease in intake (Table 4, last line). Glucosamine plus an equivalent amount of NaCl did not alter intake.

Although intake was depressed when the effluent liquid from a silo was placed in the rumen, its mode of action or constituents responsible for ite effect are not well delineated. Intake was not materially altered when lactic, acetic, or propionic acids were similarly placed in the rumen. The amounts used were approximately equal to the quantity of acids consumed by heifers when eating 15 lb. of dry matter from silage. However, when a larger amount of acids was fed there was an indication of some depressing influence on intake. It appears

unlikely that amino acids or partially hydrolyzed casein had any effect on intake, but the role of inorganic nitrogen needs further clarification.

Group C Experiments—Effect of silage constituents on silage consumption: (C-1 Intraruminal administration of silage constituents). Neutralized lactic acid (288 g.) given into the rumen did not alter intake (Table 5, Line 1).

TABLE 5

Intake of silage as influenced by intraruminal administration of various compounds (Experiment C-1)

Material given	Amount	No. heifers	No. trials	Length	Silage D.M. intake			
					Before	During	After	Change
				(days)	*(lb/heifer/day)*			
Lactate	228 g.	2	2	2	12.6	12.5	12.9	− .2
Lactate	575 g.							
Acetate	252 g.	2	2	3	12.1	8.0	10.0	−3.0
Propionate	100 g.							
Sucrose	1 lb.	2	2	3	10.7	11.4	11.7	+ .1
Water	48 lb.	1	1	3	11.9	12.1	11.5	+ .4
Tyramine	....	1	1	1	11.0	11.5	11.4	+ .3
Glucosamine	1 lb.	2	3	3	11.8	8.7	10.6	−2.5
Glucose plus NH_4OH	1 lb. & 170 g.	2	2	3	12.2	7.7	11.6	−4.2
NH_4OH	170 g.	2	2	3	11.4	4.6	9.1	−5.6
NH_4OAc	197 g.	2	4	3	11.9	9.5	11.2	−2.0
NH_4Cl	155 g.	2	2	1	12.8	11.8	.....	−1.0
$NH_4H_2PO_4$	276 g.	1	1	3	11.7	9.1	10.0	−1.8
Commercial urea	200 g.	1	1	3	12.9	3.2	8.4	−7.4
Silage juice	48 lb.	1	1	2	11.4	8.1	10.8	−3.0
Pressed silage cake	....	1	1	2	11.3	5.6	15.7	−7.9

However, when a larger quantity of a mixture of three silage acids (neutralized) was given, there was a decrease in intake (Table 5, Line 2). This decrease was comparable to that obtained when the two acids were given to heifers fed hay (Table 4, Line 14). The amounts added were approximately double the amount already being consumed in the silage.

The administration of sucrose, water, or a tyramine solution into the rumen produced no change in intake of silage (Table 5, Lines 3 to 5).

Administration of glucosamine caused some reduction in intake. An equivalent amount of nitrogen as NH_4OH with and without glucose caused a marked decrease in intake. This same amount of nitrogen as ammonium salts consistently reduced intake to a small extent. This amount of nitrogen as urea produced a large decrease in silage intake (Table 5, Lines 6 to 12).

A water extract of silage was made by adding water, macerating the silage mixture in a meat grinder, adding more water, followed by pressing with a cider press. This extract contained 4 to 5% dry matter, of which $\frac{1}{5}$ to $\frac{1}{4}$ was ash, with $\frac{1}{3}$ of the nitrogen as ammoniacal nitrogen. This silage juice caused a decrease in intake of 3.0 lb. D.M/day. The pressed silage cake was offered to

the same heifer, but she practically refused to eat it. Since both fractions caused a decrease in intake, definite conclusions are not warranted.

(C-2 Effect of mixing silage constituents with silage.) In these trials, various compounds that were likely to be normal silage constituents were added to silage when fed to heifers. The effect on voluntary intake of silage D.M. was noted.

When 575 g. lactic acid (from 2.6 lb. of a soluble Ca-Na lactate mix, Sheffield Chemical Co., Norwich, N. Y.) was mixed with wilted silage, a slight decrease (−0.24 lb.) in consumption of silage D.M. occurred. A further trial, using a Latin-square design and adding lactate, acetate, propionate, and all three acids to the silage was performed. Three animals were used in the trial. The intake decreased with all treatments (Table 6, Lines 1 to 4). The decrease

TABLE 6

Effect on daily silage intake when various materials were mixed with the silage before feeding (Experiments C-2, D-2, D-3, D-4, and D-5)

Material added	Amount	No. heifers	No. trials	Length	Silage D.M. intake			
					Before	During	After	Change
				(days)	*(lb/heifer)*			
Lactate	575 g.	3	3	3	13.3	11.7	12.4	−1.1[a]
Acetate	250 g.	3	3	3	12.4	12.3	12.9	− .3[b]
Propionate	100 g.	3	3	3	14.0	13.3	13.3	− .3[b]
All three combined	.....	3	3	3	13.0	11.1	12.8	−1.8[a]
Thyroprotein	1.0 g/cwt	1	1	2 mo.	10.1	4.9	7.6	−3.9
Thiouracil	0.5 g/cwt	1	1	2 mo.	8.6	6.1	8.2	−2.3
Limestone	¼-½ lb.	3	1	5 mo.	6.0	8.6	.....	.....
Control	0	8	1	5 mo.	6.5	8.4	.....	.....
Molasses	¼ lb.	3	1	7 mo.	5.5	6.3	8.3	.....
Control	0	9	1	7 mo.	5.1	6.2	7.3	.....
Molasses	¼ lb.	2	1	2.5 mo.	11.8	11.6	12.8	− .7
Control	0	9	1	2.5 mo.	11.5	10.4	12.4	−1.5
Molasses	¼ lb.	1	4	10 da.	14.8	14.7	15.2	− .3
Flavor Compound	45 g/cwt	6	1	10	14.0	13.0	13.3	− .6
Flavor Compound	45 g/cwt	4	1	50	7.1	9.0	12.1	− .6
0	0	4	1	50	6.7	7.3	8.6	− .3
Hay	.....	28	1	8–21 mo.	.....	19.9	.....	.....
Silage + Conc.	2 lb.	13	1	8–21 mo.	.....	11.3	.....	.....
Silage	.....	19	1	8–21 mo.	.....	11.4	.....	.....
Hay	.....	28	1	8–21 mo.	.....	11.7	.....	.....
Silage + Conc.	2 lb.	24	1	8–21 mo.	.....	8.3	.....	.....
Silage	.....	19	1	8–21 mo.	.....	8.9	.....	.....
Soybean meal	1 lb.	5	1	60 da.	7.6	9.0	10.4	± .0
Control	0	5	1	60	7.1	8.4	8.8	− .4
Aureomycin	.....	8	1	60	8.1	10.0	11.3	+ .3
Control	0	8	1	60	10.3	10.3	12.4	−1.0

Averages with the same letter as superscript form a statistically homogeneous group, P = 0.05.

was more ($P = 0.05$) when lactate or all three acids were given than when only acetate or propionate was given. The decrease in intake was somewhat less when the three acids were added to the silage than when the same amounts were given intra-ruminally (compare with Table 5, Line 2, or Table 4, Line 14).

The soluble lactate mix (2.6 lb/day, 50% lactic acid) was added to the wilted silage for a 2-mo. period for one heifer. Intake and growth rate was not depressed when compared to contemporary heifers. Calcium propionate (161 g.) was added to silage fed to two heifers for one month. Intake or growth rates were not appreciably altered.

Silage intake was reduced when large amounts of the usual silage acids, ammonium hydroxide, urea, and aqueous extract of silage were put directly into the rumen. Similar administration of other ammonium salts or compounds consistently produced a slight decrease in intake. The mode of action of these materials is not known and whether these or related compounds in silage have a similar action needs further investigation.

D: *Effect of adding various supplements to the silage on its consumption. (D-1 Effect of ammonium compounds).* Equivalent amounts of nitrogen (40 g.) as $NH_4H_2PO_4$, NH_4OAc, urea, and glucosamine were added to the silage fed for a six-day period. Each material was fed to three heifers. The average changes in silage D.M. intakes were −.3, −.1, +.6, and −1.2, respectively, compared to +.1 for the control heifers. Glucosamine was the only one of these four compounds that consistently caused a decrease in intake when added to the silage.

(D-2 Effect of thyroidally active materials). It has been postulated that there was a change in the activity of the thyroid gland of animals receiving silage (8). Thyroprotein and thiouracil were each fed to a heifer for a 2-mo. period from 8 to 10 mo. of age. Intake was drastically reduced with both treatments, compared to contemporary heifers, Table 6, Lines 5 and 6. Body weight gain was also drastically reduced. The treated heifers appeared less thrifty than heifers fed only silage. Thus, both growth rate and intake of silage-fed heifers were seriously reduced by the addition of a thyroidally active material or a thyroid depressant to the silage.

(D-3 Effect of minerals.) The acetate and carbonate salts of a mixture of Ca, Mg, K, Na, and Fe were fed to one heifer for 2 mo. and to another heifer an equivalent amount of the $PO_4^{=}$, $SO_4^{=}$, Cl^{-} salts was fed. For each 2.5 lb. silage fed, 0.1 equivalent weight of the salt mixture was added. Intake and growth rates of these heifers were slightly decreased when compared to untreated contemporary heifers.

Limestone was added to the silage of three heifers for a 7-mo. period; 0.5 lb. per day was fed to one Holstein heifer and 0.25 lb. was fed to two Jersey heifers. Average daily D.M. consumption was not altered by the addition of the limestone. For the treated Jersey heifers it averaged 6.5 lb/day, compared to 6.8 for four untreated heifers and 12.8 vs. 11.6, respectively, for the Holstein heifers. Average intakes during treatment are given in Table 6, Lines 7 and 8. Gain was less for the treated Jersey heifers (0.38 vs. 0.62 lb/day), but the same for treated and control Holstein heifers (0.93 vs. 0.95).

(D-4 Effect of flavor materials.) Molasses (0.25 lb. per day) diluted with a small amount of water was poured over the silage of heifers in three trials. The silage D.M. intakes before, during, and after the trial period are given in Table 6, Lines 9 to 13. In the first and third trials, no beneficial effect on intake was noted. In the second trial there may have been some increase compared to the control heifers; however, in this trial the heifers were fed hay during the period before treatment.

A commercial flavoring compound that has been proposed and sold as a silage additive was mixed with silage 12 hr. before feeding. This compound did not alter intake in either of two trials (Table 6, Lines 14 to 16).

In a preliminary trial, 1.0 to 1.5 lb. sugar was added to the silage. There was a slight increase in gain during the 3-mo. experimental period, but no noticeable change in silage D.M. consumed. Further trials showed that sugar did not produce any increase in silage intake of three heifers during a 2-mo. period.

(D-5 Effect of other materials). In previous trials (5, 7) a concentrate mix was fed with silage with a resulting increase in growth rate. The D.M. intake of these heifers was compared to that of 28 contemporary heifers that received only alfalfa hay and 19 heifers that received only silage. Dry matter consumption from silage when concentrates were fed was slightly less than when silage only was fed (Table 6, Lines 17 to 22). The improved performance obtained when grain was fed with silage is a result of the D.M. from the grain and not due to an increase in silage consumption. The effect of adding a similar amount of grain to the forage when put into the silo is not known, but may have a similar or more helpful effect.

One pound of soybean meal was placed on the silage of five heifers for a 2-mo. period. Gain was increased (1.1 lb/heifer/day compared to 0.5 lb. for the contemporary control heifers). However, intake of silage was not altered by the feeding of soybean meal (Table 6, Lines 23 and 24).

Potassium orotate and methionine (1 g. each day) were fed to four heifers for a period of 3 mo. without noticeably affecting rates of intake and gain.

An aureomycin—Vitamin B_{12} feed supplement [2] was added to the silage fed to eight heifers for a 2-mo. period. Gains were approximately the same (1.1 lb/heifer per day for treated compared to 1.0 lb. for contemporary control heifers). Intake may have been increased slightly by the feeding of this material (Table 6, last two lines).

In these trials, several materials were added to alfalfa silage to measure their effect on voluntary consumption of the silage by dairy heifers. Minerals, molasses, a commercial flavor material, concentrates, and an aureomycin feed supplement had no appreciable effect on silage intake. Glucosamine, lactic acid, thiouracil, and thyroprotein caused some decrease in intake of silage.

These trials have been an attempt to delineate factors that might have some effect on the voluntary intake of heifers and offer an explanation for their low intake of silage.

[2] Aurofac, containing 1.8 g. chlortetracycline and 1.8 mg. vitamin B_{12} activity/pound.

Other than the role of dry matter content of the forage, these trials were of an exploratory nature and offer limited information concerning these materials and their role in relation to intake of silage by animals. However, further investigations appear justified.

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DIGESTIBILITIES AND DIURNAL EXCRETION PATTERNS OF SEVERAL CARBOHYDRATES FED TO CALVES BY NIPPLE PAIL[1, 2]

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SUMMARY

Four calves were employed, over the age period from 3 to 6 mo., to determine the apparent digestibility of seven carbohydrates. The calves were fed milk by nipple pail at a rate of 3 lb. per 100 lb. body weight daily. The carbohydrates were added to the milk at a level of 10%. Digestion coefficients for lactose, maltose, sucrose, amylose, amylopectin, Flojel, and tapioca starch were 94, 97, 57, 83, 89, 80, and 80%, respectively. The data suggest that disappearance of considerable sucrose and starch resulted from microbial fermentation in the lower part of the digestive tract. Essentially all of the soluble carbohydrate (about one-fourth of the total carbohydrate) in the feces of starch-fed animals was glucose.

Responses in blood reducing sugar to ingestion of various carbohydrates agreed quite closely with digestibility data; the greatest increases resulted from lactose and maltose, the least from sucrose, and starches were intermediate.

The diurnal pattern of carbohydrate in the fecal dry matter (based on samples collected every 2 hr. during a 24-hr. period) exhibited two peaks which were related to the time of feeding. Peaks for lactose, maltose, sucrose, and starch (all starches combined) occurred about 10, 8, 6, and 12 hr. after feeding, respectively. Estimated digestion coefficients (calculated from diurnal samples) for lactose, maltose, sucrose, and starch (all starches combined) were 95, 96, 32, and 80%, respectively.

The ability of the calf to utilize various carbohydrates introduced posteriorly to the rumen-reticulum is not completely delineated, despite many studies which have attempted clarification. A number of different approaches have been employed in such investigations, including digestibility, growth, and blood reducing sugar studies.

Almost complete apparent digestion of lactose was obtained in digestion trials with young calves (19). Similar observations have been made with glucose and maltose in 9-mo.-old calves (12). Work at Iowa State University showed that starch and sucrose were poorly digested in calves from birth to 6 wk. of age (9). In several other studies, starch has been shown to be poorly digested by young calves (less than about 3 wk. of age); however, digestibility seemed to improve with increasing age (15, 16, 23). In another study, however, very little utilization of starch was reported in 9-mo.-old calves (12).

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Based on growth studies, lactose appears to be the carbohydrate of preference for use in milk replacers for young calves (8, 15), whereas growth responses to the feeding of glucose have proved variable (7, 8, 14, 26). However, diarrhea has resulted from feeding excessive amounts of either of these carbohydrates (7, 8, 14, 19, 22). The inclusion of starch in milk or milk replacers for young calves has almost invariably resulted in poor growth and digestive disturbances (7, 9, 15, 17). There is some indication that growth of the calf on high-starch rations improves after the first few weeks of life (15), but this has not been clearly established.

There have been several recent studies in which blood reducing sugar responses to ingestion of the various carbohydrates (when introduced posteriorly to the rumen) have been determined. With a few exceptions, the results of these studies are in agreement with the previously mentioned digestibility and growth studies. Negligible utilization of starch by the calf has been indicated by the absence of appreciable responses in blood reducing sugar (4, 5, 7, 11, 12, 18). This has been observed even in 2-yr.-old animals when the rumen is by-passed (11). On the other hand, in digestibility trials, the disappearance of considerable starch has been reported (15, 16, 20, 21, 23).

The objectives of the present study were to determine the digestibility of several carbohydrates in the young bovine (3 to 6 mo. old) when fed by nipple pail (to escape fermentation in the rumen) and to employ these digestion studies to assess the efficacy of using blood reducing sugar values for determining carbohydrate utilization.

EXPERIMENTAL PROCEDURE

Animals and treatments. Four male dairy calves (two Holsteins and two Brown Swiss) from the Iowa State University dairy herd were assigned at 12 to 14 wk. of age to a series of eight diets (Table 1). For Calf 4850, the order of the eight treatments was determined by randomization. For Calves 4851, 4857, and 4858 the rotations were started with Treatments 3, 5, and 7, respec-

TABLE 1

Sequence of feeding experimental diets [a]

Animal No.	Order of carbohydrates							
	1	2	3	4	5	6	7	8
4850	Control	Amylopectin	Maltose	Flojel	Amylose	Lactose	Tapioca	Sucrose
4851	Flojel	Amylose	Lactose	Tapioca	Sucrose	Control	Amylopectin	Maltose
4857	Amylose	Tapioca	Sucrose	Control	Amylopectin	Maltose	Flojel	Lactose
4858	Tapioca	Control	Amylopectin	Maltose	Flojel	Amylose	Lactose	Sucrose

[a] Amylose (National 78-1070), amylopectin (Amioca), Flojel 60 (thin boiling starch), and tapioca starch (refined tapioca) were supplied by National Starch Products, Inc., Plainfield, New Jersey; lactose was supplied by Western Condensing Company, Appleton, Wisconsin; maltose (D-maltose, technical grade) was purchased from Pfanstiehl Laboratories, Inc., Waukegan, Illinois; and the sucrose (granulated table sugar) was purchased from a local food store.

tively, of the rotation established for Calf 4850. However, because of a refinement in the procedure involving the disaccharides, the need for which became obvious after the first two treatments for each calf had been completed, it was deemed advisable to repeat the disaccharide diets which appeared during the first two treatment periods. Thus, maltose, lactose, and sucrose appear in Position Eight for the last three calves rather than in Positions One, Two, and Two, respectively, as originally designed.

The experimental diets consisted of alfalfa pellets (steam compressed with no binding agents), fed free choice; whole milk, fed by nipple pail at a level of 3 lb. daily per 100 lb. body weight; and carbohydrate, added to milk at a level of 10% of the milk weight (approximately 0.7 g. per pound body weight per feeding). Animals received each of the diets for ten days. The first five days constituted a period of adjustment to the ration and the calves remained in their pens; the remaining five days were spent in digestion stalls.

Prior to the experiment, the calves received a limited amount of milk replacer and grain, with hay fed ad libitum.

Collection of feces and blood. During the period in the digestion stall, all feces were collected. At 12-hr. intervals, after weighing and thorough mixing, 10% (by weight) of the feces was placed in a freezer set at about −25° C. The two collections for each calf each day were composited. These samples are referred to as composite samples.

By mechanically stimulating defecation a total of 13 fecal samples (weighing about 25 g.) were taken at 2-hr. intervals during the last 24 hr. of each five-day collection period. These samples (designated as diurnal samples) were stored at about −25° C. for subsequent analyses.

On the fifth day in the digestion stalls, blood was sampled immediately prior to the morning feeding and at 1, 2, 3, 4, 6, and 8 hr. thereafter and analyzed for reducing sugars. Preparation of a protein-free filtrate and analysis for reducing sugar were according to the method of Nelson (13). These data were analyzed statistically, employing a multiple range test (6).

Analyses of feces. Feces were thawed at room temperature, thoroughly mixed, and aliquots taken for determination of dry matter and carbohydrate content. Separate analyses were made for each of the five daily composite samples and for each of the diurnal samples. Per cent dry matter was determined on about 8 g. of feces, according to the method used by Owen (19).

Carbohydrate in the feces of lactose- and maltose-fed animals was determined as follows: (1) Five grams of feces were thoroughly mixed with water, the mixture was centrifuged and a protein-free filtrate was prepared from the supernatant, according to the Nelson method (13) for blood. (This method was also used for preparation of protein-free filtrates in other analyses, as well as in the determination of reducing sugar.) (2) The extract then was used for preparing ascending paper chromatograms according to the procedure of Dimler *et al.* (3), with the following modifications: (a) Ten microliters of solution were placed on the paper in 5-μl. aliquots. (b) The solvent system contained 1-butanol, pyridine, and water (6:4:3). (c) The dip method described by

Trevelyan *et al.* (24) was used for developing guide strips. (d) Rather than eluting carbohydrate from chromatograms, small squares of paper (uniform in size) containing the carbohydrate spot were cut into small pieces and placed directly into the copper reagent for determination of reducing sugar content (13). Squares with no added carbohydrate were used as blanks. Chromatograms showed only spots corresponding to glucose and galactose, indicating complete hydrolysis of lactose and maltose prior to analysis.

Sucrose in the feces of sucrose-fed animals was determined as follows: Five grams of feces were thoroughly mixed with 84 ml. of water. Eight milliliters of each of the protein-precipitating reagents (13) were added and the resulting mixture was filtered. Four milliliters of the filtrate were diluted with 96 ml. of water and reducing sugar content of a 2-ml. aliquot was determined before hydrolysis (13). Sucrose in the remaining filtrate was hydrolyzed by incubation for 2.5 hr. with an invertase solution,[4] according to the method presented by A.O.A.C. (1). Following incubation, content of reducing sugar was determined on 2-ml. aliquots of incubated solution.

Procedures for analyzing feces of animals on the control and starch rations were identical. Five grams of feces were mixed with 25 ml. water and the mixture was centrifuged at 14,000 × gravity for 20 min. A protein-free filtrate (13) was prepared from the supernatant (which contained soluble carbohydrate). This protein-free filtrate was analyzed for content of reducing sugar. (Chromatograms showed that nearly all of the carbohydrate in this protein-free filtrate was glucose.) The nonsoluble carbohydrate (present in the residue) was hydrolyzed according to the A.O.A.C. method (1). After centrifugation, the hydrolyzed extract was neutralized and a protein-free filtrate was prepared. The filtrate was then chromatographed and content of reducing sugar was determined according to the method previously described. In samples from starch-fed animals, two distinct spots appeared on the chromatograms. One corresponded to glucose and the other to a pentose (probably xylose). In daily composite samples, reducing sugar activity of each spot was measured separately whereas, in diurnal samples, only the spot corresponding to glucose was determined, because the pentose undoubtedly originated from the alfalfa pellets.

RESULTS AND DISCUSSION

Digestibility data are presented in Table 2. The high apparent digestion values for lactose and maltose are in accordance with other studies (9, 10, 12, 19) and greatly exceed the values for most of the other carbohydrates in the present study. The superiority of these carbohydrates to others tested was also shown in blood reducing sugar responses (Table 3). The results of the present study agree with those of an earlier experiment (11), in which it was shown that apparent utilizations of lactose and maltose were similar in calves older than 3 mo. However, diarrhea resulted from ingestion of lactose and maltose in the former study, but not in the present investigation. This may be attributed, at

[4] A 1% (w/v) aqueous solution of invertase scales. The enzyme was obtained from Wallerstein Co., Staten Island, New York.

TABLE 2

Apparent digestion of supplemental carbohydrates [a, b]

	Carbohydrate						
	Lactose	Maltose	Sucrose	Amylose	Amylopectin	Flojel	Tapioca
Intake per day *(g.)*	391	375	401	302	340	327	317
Output in feces per day *(g.)*	22.1	11.3	173	52.7	39.0	64.3	64.4
Carbohydrate recovery in feces							
Per cent recovered	6	3	43	17	11	20	20
Standard error of the mean	1.3	1.7	12.2	5.0	8.5	9.6	9.0
Coefficient of digestion *(%)*	94	97	57	83	89	80	80

[a] Apparent digestion of lactose of whole milk was almost complete; therefore, recovery data for the control ration are not presented. However, these data were used in calculating recovery of supplemental carbohydrate by subtracting the grams of carbohydrate recovered in the control ration from the grams of carbohydrate recovered in each supplemented ration.
[b] Each value is the mean of four animals (averaged for a five-day period).

least in part, to the higher level of intake at each feeding in the earlier study (2 g. per pound body weight), resulting in a hydragogue effect, than in the present study (about 1 g. per pound body weight). It is possible, also, that insufficient lactase and maltase were available for complete digestion of the carbohydrates at the higher level of feeding.

Even though digestion coefficients for sucrose were much lower than for the other carbohydrates, they were higher than expected. Blood reducing sugar (4, 5, 11, 18, 25) and tissue enzyme studies (4, 5, 9, 10) have invariably indicated that the bovine has no intestinal sucrase. Disappearance of considerable sucrose was believed to have been caused by fermentation by microorganisms in the lower digestive tract and in the feces after excretion. This was indicated

TABLE 3

Effect of ingestion of carbohydrate (via the milk) on changes in levels of blood reducing sugar

	Carbohydrate [a, b]								Standard error of treatment mean
	Control	Sucrose	Amylopectin	Flojel	Tapioca	Amylose	Lactose	Maltose	
	(mg. %)								
Maximum increase [c]	19.6	13.3	19.0	24.9	25.3	26.3	36.9	46.5	4.4
Mean change [d]	1.5	2.5	7.1	6.6	8.6	14.1	14.0	14.2	1.6

[a] The lactose contained in whole milk was equivalent to about 0.35 g. per pound body weight per feeding. Supplemental carbohydrate was fed at a level of about 0.7 g. per pound body weight per feeding.
[b] Any two means not underscored by the same line are significantly different.
[c] Greatest positive change in blood reducing sugar due to carbohydrate ingestion. (Each value is the average of four animals.)
[d] Average change in blood reducing sugar taken at 1-hr. intervals for the 5-hr. period subsequent to ingestion of carbohydrate. (Each value is the average of four animals.)

by a much lower digestion coefficient for sucrose for diurnal samples, 32, (placed in freezer immediately after being voided) than for composite samples, 57, (accumulated for 12 hr. before freezing). Carbohydrate excreted in the diurnal samples was estimated by multiplying the carbohydrate content (per cent) of the fecal dry matter (averaged for 13 collections during a day) by the total quantity of dry matter voided. From this value and carbohydrate intake, the apparent digestibility was derived.

Digestion coefficients calculated from diurnal samples for lactose, maltose, and starch (all starches combined) were 95, 96, and 80%, respectively; those determined from composite samples were 94, 97, and 83%, respectively. This close agreement indicates that there was little fermentation of carbohydrate (other than sucrose) during the period that feces remained in the collection pans, and suggests that digestibility coefficients from the diurnal data were valid estimates of carbohydrate digestion, although the existence of compensating errors is possible.

Extensive microbial hydrolysis of sucrose was indicated by the appearance of 68% of the carbohydrate as monosaccharide in composite samples and 38% in diurnal samples (Table 4). There is also evidence that some of the starch

TABLE 4

Monosaccharide content of carbohydrate voided in composite and diurnal samples of feces [a]

	Carbohydrate fed					
	Sucrose	Amylose	Amylopectin	Flojel	Tapioca	Av. of all starches
	(%)					
Composite samples	67.5 [b]	18.0 [b]	36.1 [b]	23.3 [b]	23.2 [b]	25.2
Diurnal samples	38.0 [c]	20.7 [d]	38.0 [d]	22.3 [d]	30.8 [d]	28.0

[a] All of the small amount of hexose carbohydrate in the feces of animals receiving lactose, maltose, and control rations was monosaccharide; therefore, data are not presented for these rations.

[b] Average of four animals. Value for each animal is an average of five days.

[c] Average of four animals. Value for each animal is an average of 13 diurnal samples.

[d] Average of three animals. Value for each animal is an average of 13 diurnal samples.

was hydrolyzed by microorganisms in the lower digestive tract and in the feces after excretion. Carbohydrate appearing as monosaccharide in composite and diurnal samples from starch-fed animals was 25 and 28%, respectively.

It is interesting to note that essentially all of the soluble carbohydrate in feces of starch-fed animals was glucose, with only traces of dextrin and maltose (as evidenced by chromatographic analysis). In many of the earlier studies, the A.O.A.C. method was used for assessing the starch content of feces. In this method, feces are washed and all water-soluble substances are discarded. It has been shown in the present study that such a procedure might lead to erroneous conclusions regarding the utilization of starch, because about one-fourth of the recovered carbohydrate was in the soluble form.

Digestion coefficients for starches (ranging from 80 to 89%) were quite similar to those reported by Noller *et al.* (15, 16) and Ratcliff (20). The

apparent discrepancy between the present data and those of Larsen *et al.* (12) may be due partially to a difference in methods. The digestibilities reported herein were based on the total collection of feces for a five-day period, whereas the method used by Larsen and associates (12) employed the ratio technique, with chromium oxide as a marker. Sampling times were 8 and 10 hr. after feeding. Different rates of passage of the marker and starch in the latter could have resulted in an error in the calculated digestion coefficients. The higher starch digestibilities in the present study may also have been due partially to a longer time for adaptation of animals to starch-containing rations. The test diets employed by Larsen *et al.* (12) were fed for only two days as compared to a ten-day feeding period in the current experiment. However, if such adaptation did occur, it probably was complete before the collection period began, because no increase in digestibility of starch was noted during the collection period. Digestibility of starch in the study of Larsen *et al.* (12) may also have been influenced by the fact that animals were fitted with rumen and cecal fistulas and the rumen was completely evacuated previous to introduction of the test meal.

Mean changes, the calculation of which was described previously (11), in blood reducing sugar were significantly higher ($P < .05$) for animals receiving starch rations than for the controls, while the differences in maximum increases were not significant (Table 3). Increases due to starch ingestion were more persistent than those for the controls and indicate that breakdown of starch occurred at a slower rate than that of lactose (contained in milk). Because they also reflect persistency of the glucose tolerance curve, the mean changes probably are more meaningful than maximum increases as estimators of starch utilization.

In an earlier experiment (11) negligible responses in blood sugar were shown when starch was fed in a water suspension. Possible reasons for the greater apparent utilization in the present study are: (1) Milk, in which the starch was suspended, may have stimulated a greater release of pancreatic enzymes. It also may have resulted in more efficient by-passing of the rumen. (2) Starch was fed for a longer period of time and may have resulted in greater amylase production due to enzyme adaptation.

The dry matter content of the feces of animals on sucrose diets was somewhat lower than for other diets (Table 5). Per cent dry matter in the feces of animals receiving disaccharide diets also was related to the time of the previous feeding and the most fluid feces usually were passed at the hour when carbohydrate content in the feces was highest (Table 6). For example, average dry matter and carbohydrate (of the dry matter) in the feces just prior to the morning feeding of a test meal of sucrose were 17.3 and 0.3%, respectively, whereas the corresponding values in fecal samples 6 hr. later were 13.5 and 43.1%.

The carbohydrate content of the fecal dry matter reached a maximum for sucrose-fed animals at about 6 hr. after feeding (Table 6). Maxima for animals receiving maltose, lactose, and starch were at about 8, 10, and 12 hr., respectively. This high degree of diurnal variability in carbohydrate content of feces indicates that caution must be used in application of the ratio technique in estimat-

TABLE 5

Dry matter content of diurnal samples of feces

	Time												
	A.M.			P.M.						A.M.			
Treatment	7[a]	9	11	1	3	5[a]	7	9	11	1	3	5	7
	(%)												
Control	20.2	21.4	23.0	21.4	21.8	21.4	20.3	21.0	21.1	21.1	21.4	20.9	20.9
Lactose	20.0	21.2	21.3	19.8	19.8	18.1	20.6	20.4	20.9	19.0	18.2	20.3	20.1
Maltose	18.0	17.5	16.9	18.4	15.0	16.3	18.8	17.9	17.2	15.4	15.0	16.1	18.8
Sucrose	17.3	18.5	14.3	13.5	14.2	12.5	14.4	14.3	13.1	13.1	13.7	15.1	18.1
Starches[b]	19.8	19.7	20.0	20.6	21.3	21.0	19.9	20.0	20.1	20.5	19.8	19.5	19.4

[a] Time of feeding milk (containing carbohydrate) by nipple pail.
[b] Because differences were small, values for the four kinds of starch were combined.

ing the digestibility of a substance fed to the calf by nipple pail. This would be especially true if digestion coefficients were based on analyses made from a few grab samples of feces. Small differences in the rate of passage of the marker and nutrient also might result in erroneous conclusions.

An average of 64 g. of pentosan per day was recovered in the feces of animals receiving starch and control diets. (Quantity of pentosan was not determined in feces of animals receiving disaccharides.) Since similar amounts were recovered in control and starch diets, it appears that the pentosans came from the forage portion of the diet. This conclusion is supported by the appearance of only one spot (corresponding to a pentose) on chromatograms of hydrolyzed feces from control animals. Barhydt and Dye (2) reported that 40 to 50 g. per day of pentosan escaped fermentation in 5-mo.- to 1-yr.-old calves on a hay-grain diet.

Future investigations are needed to assess quantitatively the extent of starch and sucrose hydrolysis by intestinal microorganisms. This would permit more accurate evaluation of the contribution of these carbohydrates to nutrition of the calf, when the feeding system is such that the carbohydrates by-pass the rumen.

TABLE 6

Carbohydrate content of dry matter in diurnal samples of feces[a]

	Time												
	A.M.			P.M.						A.M.			
Treatment	7[b]	9	11	1	3	5[b]	7	9	11	1	3	5	7
	(%)												
Lactose	0.6	0.3	0.4	2.7	3.4	5.2	2.3	0.8	1.8	4.1	4.7	1.3	0.7
Maltose	0.6	0.3	....	0.4	4.0	2.2	0.9	0.7	2.2	4.2	4.3	1.9	1.0
Sucrose	0.3	0.2	12.5	43.1	38.4	29.0	17.6	7.8	30.9	29.2	22.1	9.9	2.1
Starches[c]	4.2	3.3	1.4	1.4	3.0	9.8	14.1	10.2	2.8	4.1	8.8	11.4	7.7

[a] This table includes only values for hexose carbohydrate. Controls are not listed because of the presence of negligible amounts of hexose carbohydrate.
[b] Time of feeding milk (containing carbohydrate) by nipple pail.
[c] Because of the small differences between them, values for the four kinds of starch were combined.

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DIGESTIVE ENZYME ACTIVITIES IN THE YOUNG CALF[1, 2]

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SUMMARY

Twelve male calves were divided into comparable groups at birth. One calf from each group was sacrificed at each of the following ages: 1, 8, 15, 22, 33, and 44 days. Fourteen hours prior to sacrifice, calves from Group I received a test meal of whole milk plus 3% lactose and Group II received whole milk plus 2.5% sucrose and 0.5% starch. Except for calves sacrificed at one day, diets similar to the test meal were given to calves from four days of age to time of sacrifice.

After sacrifice, certain organs were removed and analyzed for digestive enzyme activities. Intestinal lactase activity was highest at one day of age and decreased thereafter. Intestinal maltase levels were low (as compared to lactase) and did not change with age. No intestinal sucrase was detected. Gastric protease was highest at eight days and decreased thereafter. Pancreatic amylase, lipase, and protease were lowest at one day, increased during the first week, and changed little thereafter. Diet apparently exerted no major effect on levels of any of the digestive enzymes.

The developing rumen of the young calf has, in recent years, been a major area of research interest; however, there have been relatively few studies to characterize the digestive tract of the calf posterior to the rumen. Studies with milk substitutes have shown that, during the early post-natal period, the calf possesses only limited ability to digest certain common feeds, especially of plant origin (5, 19). Both qualitative and quantitative differences between the digestive enzyme complex of the calf (10) and that of simple-stomached animals (7) have been noted. These studies also have pointed to a change with age in the ability of the calf to utilize certain nutrients.

The purpose of the present investigation was to determine the effect of age and diet upon activities of the major digestive enzymes in calves sacrificed at intervals up to 6 wk. of age.

EXPERIMENTAL PROCEDURE

Animals. Twelve male dairy calves (nine Holsteins and three Ayrshires) were paired on the basis of time of birth; each pair was randomly assigned to a sacrifice age (1, 8, 15, 22, 33, or 44 days). One calf from each pair was

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allotted to one of two dietary groups. Group I received whole milk plus 3% lactose, and Group II received whole milk plus 2.5% sucrose and 0.5% corn starch.

Calves sacrificed at one day were removed from their dams immediately after birth and thus received no colostrum. All other calves remained with their dams until three days of age, then were placed in metal pens with screen floors (no bedding). Whole milk was fed at the daily rate of 7, 8, 9, and 10% of body weight from the 1st to 4th wk. of age, respectively. After 4 wk. the 10% level was maintained. Lactose and sucrose readily dissolved in the whole milk, whereas suspension of the starch was effected by heating to 55° C. Animals received no grain or hay, but water was available up to the time of feeding the test meal.

Fourteen hours prior to sacrifice each calf received a test meal of its previous diet plus 1 to 2 g. of carmine red (to detect forward margin of the test meal). Calves sacrificed at one day received a test meal similar to that of other calves in the respective groups.

After sacrificing by a hammer blow on the head and severance of the jugular veins and carotid arteries, the entire gastrointestinal tract and the pancreas were removed from the abdominal cavity. The tract was opened and gently washed with distilled water. The abomasum, pancreas, and small intestine (divided into three sections of approximately equal length) were placed in a freezer at about −25° C., where they were stored until analyzed.

Determination of enzyme activities. Frozen intestine was chopped into small pieces and comminuted in a Serval Omnimixer (1,400 r.p.m. for 1 min.) with four times its weight of water. These tissue homogenates were then transferred to covered Erlenmeyer flasks, layered with 30 ml. toluene, and allowed to stand at room temperature for 20 to 22 hr., during which time they were agitated frequently. Subsequently, samples were strained through cheese cloth (eight layers) and 25-ml. aliquots were transferred to incubation flasks which contained 25 ml. of a carbohydrate substrate prepared according to Heilskov (8). The substrates (lactose, maltose, and sucrose) were adjusted to pH values of 5.5, 7.0, and 7.0, respectively.

After incubation for 4 hr. at 40° C. the increase in reducing activity of the mixture (tissue homogenate and disaccharide substrate) was measured according to the Heilskov procedure (8). Enzyme activities were determined by comparing these increases to a glucose standard curve and are expressed as milligrams glucose produced per gram of dry tissue.

A weighed sample of chopped abomasum was comminuted in a Serval Omnimixer (1,400 r.p.m. for 1 min.) with ten times its weight of 0.85 *N* HCl. Homogenates were then placed in a 40° C. water bath for 18 hr., to allow conversion of pepsinogen to pepsin, after which the homogenates were strained through cheese cloth (four layers) and kept in a cold room (at 4° C.) for a short time prior to analysis.

Pepsin activity was measured by the milk-curdling test, according to a procedure adapted from that of Anderson *et al.* (1). Modifications included the

use of reconstituted skimmilk (instead of pasteurized milk) and adjustment of the pH of the milk to 5.5. Enzyme activities were determined by comparing the results from the abomasal homogenates to a standard curve (prepared with varying dilutions of granular pepsin [4]) and are reported as National Formulary (N.F.) values.

Pancreatic tissues were chopped and homogenized (as described previously) in 20 times their weight of distilled water. These homogenates were further diluted with distilled water, so that the respective portions used for determining lipase and protease activities contained 0.934 and 0.471 mg. of tissue per milliliter of homogenate. Another homogenate prepared similarly with tissue and 0.2% NaCl (1:4 dilution) was used for determining pancreatic amylase activity.

The procedure of Goldstein *et al.* (6) was modified in the following manner to determine pancreatic lipase activity: (a) After incubation, samples were titrated to pH 10.65 (as measured on a Beckman Model H-2 pH meter) with 0.0964 *N* NaOH. During titration, samples were stirred with a mechanical stirrer. (b) Lipase activity was read directly from a standard curve (prepared with Steapsin [5]). Activity of 1 ml. of 2% Steapsin solution represented 100 lipase units.

For determination of pancreatic amylase activity, 1 ml. of tissue homogenate was incubated for 30 min. at 40° C. with 29 ml. of starch substrate,[6] after which reducing sugar activity was measured according to the Somogyi method (22). Amylase activities were read directly from a maltose standard curve and are presented as milligrams maltose produced per gram of tissue.

The homogenate used for determination of pancreatic protease was mixed with enterokinase solution,[7] adjusted to pH 5.6, and placed in a cold room for two days at 4° C. to permit enzyme activation. The disc plate method employed by Lewis *et al.* (18), for determining trypsin levels in swine pancreas, was adapted for measuring pancreatic protease activity. Analyses were made with a dilution of tissue homogenate containing 1.5 mg. of streptomycin and 1.5 mg. of chlortetracycline per milliliter. There were four replications of each of three dilutions of the homogenate. Protease activities were read directly from a standard curve prepared with pancreatin.[8] (Activity of a 5% pancreatin suspension was designated as 100 protease units, per disc.)

A constant dry matter weight was determined on all tissues by heating in a drying oven at 105° C. for 30 hr. Activities of all enzymes are presented on a dry tissue basis.

[4] Granular pepsin (1:15,000 strength) obtained from Cudahy Packing Co., Omaha, Nebraska.

[5] Steapsin obtained from Wilson Laboratories, Chicago, Illinois.

[6] A phosphate buffered solution (pH 6.8) containing 0.814 mg. of Merck acid-treated potato starch per milliliter.

[7] The final mixture contained 0.25 mg. of enterokinase per milliliter of solution. Enterokinase was prepared according to Kunitz (15).

[8] National Formulary Reference Standard for Casein Digestion Power obtained from the Committee on National Formulary, Washington, D. C.

RESULTS AND DISCUSSION

The lactose-supplemented diet, in which lactose constituted about 50% of the dry matter, caused very little diarrhea (average of 0.08 per calf per day based on scale from 0 = normal to 4 = severe diarrhea), whereas a high incidence of diarrhea (average of 1.35) was noted among the sucrose-starch group. Other workers have shown similar results when either sucrose or starch has been included in liquid diets for young calves (5, 19, 20, 23).

There was little effect of age or diet upon wet or dry weights of digestive glands (Table 1). These weights seemed to be quite closely related to body

TABLE 1

Average weights of abomasum, small intestine, and pancreas [a]

Tissue	Av. age at sacrifice	Actual weight		Per cent body weight	
		Wet	Dry	Wet	Dry
	(days)	*(g.)*			
Abomasum [b]	1	206	46	0.50	0.11
	8	194	40	0.53	0.11
	15	172	41	0.44	0.10
	22	232	49	0.53	0.11
	33	180	35	0.49	0.10
	44	228	62	0.44	0.12
Small intestine [b]	1	982	198	2.39	0.48
	8	852	164	2.34	0.45
	15	801	163	2.05	0.42
	22	990	182	2.27	0.42
	33	956	182	2.60	0.49
	44	928	200	1.78	0.38
Pancreas	1	34	11.3	0.08	0.03
	8	25	7.3	0.07	0.02
	15	22	7.1	0.06	0.02
	22	33	8.9	0.08	0.02
	33	27	10.6	0.07	0.03
	44	53	17.2	0.10	0.03

[a] Each figure is an average of two calves (one on each diet). These averages were used, since diet had no detectable effect on organ weight.
[b] Weights of empty abomasum and small intestine.

weights of animals. The average per cents of body weight of abomasum, small intestine, and pancreas were 0.48, 2.24, and 0.08 (on a wet weight basis), respectively.

Lactase activities were highest at one day of age, decreased to about one-half the original values by 22 days, and changed little from 22 to 44 days (Table 2). A similar decrease with age of calves has been reported by other workers (4, 9); however, little change in lactase levels in sheep from 1 to 5 wk. of age was noted (25). It seems logical that lactase should be high during early life, because of the importance of milk as a dietary constituent. The decrease with age in lactose utilization, as reported in other studies (4, 11), may be partially explained by a decrease in lactase activity of the small intestine. A similar decrease with age in the lactase activities in young pigs has been observed; however, at birth the

TABLE 2

Enzyme activities of the various organs [a]

Diet	Av. age *(days)* 1	8	15	22	33	44	Av.
			Intestinal lactase *(mg. glucose)*				
Sucrose-starch	1,549	1,233	980	550	868	673	976
Lactose	1,233	592	939	725	562	686	790
Average	1,391	912	960	638	715	680	
			Intestinal maltase *(mg. glucose)*				
Sucrose-starch	99	82	93	70	104	81	88
Lactose	48	104	75	98	100	103	88
Average	74	93	84	84	102	92	
			Pancreatic amylase *(mg. maltose)*				
Sucrose-starch	229	771	1,229	626	635	1,463	826
Lactose	374	834	592	1,047	1,580	325	792
Average	302	802	910	836	1,108	894	
			Abomasal protease *(N. F. value)*				
Sucrose-starch	1:3875	1:5340	1:2191	1:1993	1:2402	1:1199	1:2833
Lactose	1:2177	1:3597	1:2092	1:1163	1:1826	1:1243	1:2016
Average	1:3026	1:4468	1:2142	1:1578	1:2114	1:1221	
			Pancreatic protease *(protease units)*				
Sucrose-starch	45	162	106	106	127	171	120
Lactose	39	125	138	116	55	125	100
Average	42	144	122	111	91	148	
			Pancreatic lipase *(lipase units)*				
Sucrose-starch	1,067	3,328	3,098	2,521	2,488	3,100	2,600
Lactose	1,214	3,031	2,081	3,295	1,850	2,223	2,282
Average	1,140	3,180	2,590	2,908	2,169	2,662	

[a] See text for method of assay and description of units.

activities were about five times as high (per unit of body weight) as those of the one-day-old calves (2, 7, 24).

Much of the decrease with age in lactase activity is attributable to a decreased concentration of the enzyme in the posterior third of the small intestine (Table 3). Higher lactase levels in the anterior than in the posterior areas of the small intestine of calves have been reported by Heilskov (9).

TABLE 3

Lactase activity of the segments of the small intestine (milligrams glucose) [a]

Average age *(days)*	Segment I	Segment II	Segment III
1	1,427	1,828	919
8	1,365	1,196	173
15	1,278	1,447	155
22	954	913	47
33	1,521	580	44
44	1,242	744	52

[a] The small intestine was divided into three segments of approximately equal length (Segment I, anterior third; Segment II, middle third; Segment III, posterior third). See text for method of assay and description of units. Each figure is an average of two calves.

The additional lactose in the ration of Group I apparently had little effect upon lactase levels. Perhaps the difference in quantity of lactose between the two rations was not great enough to induce differing concentrations of enzymes. It is also possible that the period of adaptation was not sufficient for induction to occur.

Maltase levels, when compared to lactase, were very low. No apparent age or dietary differences were observed (Table 2), in contrast to studies by English workers who noted greater maltase activities in 8-wk.- than in 3-wk.-old calves (4). Under the normal feeding regime, there may be little need for intestinal maltase. However, low levels of maltase and amylase may limit the quantity of starch which can be utilized efficiently in milk replacers. Other studies have shown that utilization of maltose is poor in calves of less than 4 wk. of age, but improves by about 7 wk. (4, 11, 20, 23).

Intestinal sucrase was not detected in any of the calves. Similar results have been reported by others in calves (4) and sheep (25). These findings are substantiated by several reports in which no indication of sucrose utilization in calves was shown (4, 11, 20, 23). Digestion coefficients of sucrose fed to calves were quite low and it has been suggested that much of the disappearance that did occur was due to microbial fermentation (12). In sharp contrast to the results reported for calves are those with swine, in which high levels of sucrase were shown after 2 wk. of age (2, 7). Sucrose was well utilized after pigs were ten days old (3).

Levels of pancreatic amylase were quite variable, being lowest at one day, nearly tripling by eight days, and remaining relatively constant thereafter (Table 2). Dollar and Porter (4) reported slightly greater pancreatic amylase activities in 8-wk.- than in 3-wk.-old calves. Activities in the present study are in very close agreement with those observed by the English workers (4). Amylase activity in the pancreas of the new-born pig is low, but increases rapidly up to 5 wk. of age (13, 14, 24). When blood reducing sugar measurements were used as an index of starch utilization by the calf, no positive responses were obtained (4, 11, 16, 20). In digestibility studies, however, considerable utilization of this carbohydrate by the calf has been observed (12, 19, 21). Additional studies are needed to clearly evaluate the fate of starch introduced posterior to the rumen of the young bovine.

Gastric protease levels were quite high at one day, were highest at eight days, and decreased thereafter (Table 2). On the other hand, pancreatic protease activities were low at one day and much higher thereafter. Absence of a growth response to addition of pepsin to milk replacers for calves (17, 21) may possibly be explained by the relatively high levels of abomasal proteases which normally are present. These results are in contrast with those reported for swine, in which stomach protease levels were lowest at birth and increased manyfold by 5 wk. of age (7, 18). Low pancreatic protease levels in new-born calves, together with the antitryptic factor of colostrum, probably are important in keeping immune globulins intact for absorption during the first few days of the calf's life. The relationship of the high activity of gastric protease to this phenomenon is not known.

A trend similar to those noted with other pancreatic enzymes was found with pancreatic lipase; activities were lowest at one day, increased about threefold by eight days, and changed little thereafter (Table 2). Studies with swine show slight increases in lipase activity during the first few weeks of life (7, 14). The high digestibilities of ether extract reported for calves (19) substantiate the presence of considerable pancreatic lipase.

It has been shown that tissue levels of digestive enzymes vary from birth to 6 wk. of age in the calf. However, additional studies with young animals and similar studies with older animals are needed. Investigations of the secretion rates of the different digestive enzymes would also be valuable, since tissue concentrations may not always reflect adequately the quantity of enzyme available during digestion.

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HERITABILITY, PHENOTYPIC AND GENETIC CORRELATIONS BETWEEN TYPE RATINGS AND MILK AND FAT PRODUCTION IN HOLSTEIN-FRIESIAN CATTLE [1]

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SUMMARY

Intra-sire, intra-herd variance and covariances of daughters and dams were used to estimate heritabilities, and phenotypic and genetic correlations between type ratings and milk and milk fat production records adjusted to a single record basis. Milk and milk fat production records and official type classification ratings of 14,727 Holstein-Friesian cows and their dams were included in this study.

Heritability estimates for milk and milk fat production were of the order of 0.20, and did not differ significantly among the three levels of herd production. The estimates of heritability for type ratings ranged from 0.03 for dairy character to 0.31 for rump.

Phenotypic correlations between type ratings and milk and milk fat production ranged from +0.02, to +0.25, with a standard error of 0.01 to 0.02. Most of the genetic correlations concerning type ratings and production were not significant. The genetic correlations between dairy character and production were significant and ranged from 0.61 to 0.82 for the three stratified groups.

Selection based on type ratings, with the exception of rating for dairy character, would cause little genetic improvement in milk or milk fat production. The relative genetic progress in milk production, if selection is on dairy character, is expected to be 35 to 50% of that which can be obtained by selecting on the basis of one milk production record.

The emphasis placed on type in breeding programs varies greatly among breeders. The importance of type in a selection program where the primary goal is to improve the genetic ability for a production trait will depend upon the heritabilities of type and production and the genetic correlation between these traits.

The objectives of this study were: (1) To estimate the heritability of milk and milk fat production and the official type ratings of Holstein-Friesian cattle; (2) to calculate phenotypic and genetic correlations between milk and milk fat production, among the different type components, and among production and type traits; and (3) to determine if the above statistics vary according to level of production of Holstein-Friesian cattle.

MATERIALS AND METHODS

The data for this study consisted of type ratings and milk and milk fat production records of 59,511 H.I.R. and A.R. Holstein-Friesian cows. The

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registration number of the cows and their sires and dams, herd number, coded official final type classification rating and component ratings, and an average of all milk and milk fat production records (adjusted to a 2 ×, M.E., 305-day basis) for each cow were punched in I.B.M. cards by the Holstein-Friesian Association of America. Type ratings were coded as follows: excellent, 5; very good, 4; good plus, 3; good, 2; and fair, 1. No data were available on animals with a final type classification rating of poor, since their registration papers are automatically cancelled.

This study included those animals which had completed at least one lactation record and had an official type classification rating (including components) prior to January 1, 1952. Single daughter-sire groups were removed as a part of an earlier study (15). The remaining 45,111 cards were duplicated in order to obtain daughter-dam pairs. Only daughters and their dams which made their records in the same herd were included in this study.

Regressions should be unbiased, unless there is a correlation between the size of a cow's record and the number of her daughters. This was checked and there was no indication in these data that this had occurred.

Harvey and Lush (3) demonstrated that the amount of year component in the intra-herd variance of the dam is considerably reduced by averaging all the records available on the dam. They also concluded that unless there is a deliberate effort to compare only the records which the dam and her daughter made in the same year, the year effects in the intra-herd covariance will always be small. On the basis of this evidence, no adjustments for year effects were undertaken in this analysis.

Since there were approximately 11,000 dams and 16,000 daughters, the card of each dam (having more than one daughter) was duplicated. This resulted in approximately 16,000 daughter-dam pairs. Single daughter-sire groups were eliminated, leaving 14,727 daughter-dam pairs for the analysis.

Selection of the independent variable does not affect regression, whereas selection of the dependent variable may bias the regression coefficient. Since type classification breakdown ratings were initiated in 1945, the daughters of cows which were born after June, 1940, had no more than the usual opportunity to be selected prior to classification. However, dams which were born before June, 1940, could have dropped daughters which could have been highly selected before they were classified. Because the animals in this study could have made their production records over a period of approximately 20 yr., the data were divided into two separate analyses. The first analysis (hereafter referred to as Analysis I) consisted of dams born prior to June, 1940, and their daughters. Analysis II contained the remainder of the daughter-dam pairs.

To determine if there was any influence of herd level of production on the statistics to be computed, the daughter-dam pairs in Analysis II were stratified into three groups, based on herd averages for milk production. This stratification of herds was based on the original 59,511 M.E., 2 ×, 305-day average milk yields. This total consists of all the Holstein cows that had both production and type component information prior to January 1, 1952. The three groups

were: low (< 11,960 lb. milk); medium (11,960 to 13,230 lb. milk); and high (> 13,230 lb. milk). The number of daughter-dam pairs in each group were 3,831, 3,991, and 3,548, respectively. The data in Analysis I were not stratified, and consisted of 3,357 daughter-dam pairs.

Within-sire, within-herd variances and covariances were utilized in estimating all correlations in this study. The means and within-sire, within-herd standard deviations for both daughters and dams for production and type ratings are shown in Table 1.

TABLE 1

Distribution of herds, sires, daughter-dam pairs, and the means and standard deviations for milk and milk fat production and type ratings

	Low group		Medium group		High group		Analysis I	
No. herds	263		317		297		467	
No. sires	585		652		587		853	
No. pairs	3,831		3,991		3,548		3,357	
	Dams	Daus.	Dams	Daus.	Dams	Daus.	Dams	Daus.
Milk								
Mean	11,232	11,069	12,708	12,648	14,393	14,259	12,590	12,460
St. dev.	1,860	2,020	2,026	2,082	2,097	2,312	2,205	2,367
Milk fat								
Mean	399	399	456	463	514	520	443	450
St. dev.	71	76	77	78	86	86	83	90
Final rating								
Mean	2.74	2.24	3.03	2.53	3.10	2.64	3.33	2.79
St. dev.	.92	.85	.90	.85	.87	.82	.90	.91
Gen. appear.								
Mean	2.77	2.28	3.02	2.55	3.06	2.65	3.27	2.80
St. dev.	.87	.82	.89	.84	.86	.84	.87	.90
Dairy character								
Mean	3.45	3.19	3.62	3.29	3.70	3.45	3.87	3.41
St. dev.	.82	.75	.81	.74	.81	.74	.82	.84
Body capacity								
Mean	3.29	2.91	3.49	3.10	3.57	3.17	3.85	3.28
St. dev.	.86	.81	.83	.79	.85	.80	.85	.88
Mammary system								
Mean	2.65	2.24	2.91	2.46	2.94	2.58	3.16	2.69
St. dev.	.93	.87	.94	.86	.91	.86	.98	.93
Feet and legs								
Mean	2.50	2.06	2.70	2.35	2.77	2.40	2.98	2.60
St. dev.	.97	.93	.98	.92	.96	.90	.99	.95
Rump								
Mean	2.73	2.31	2.96	2.58	3.00	2.65	3.06	2.74
St. dev.	1.11	1.08	1.05	1.04	1.07	1.04	1.11	1.08

Heritabilities were estimated by doubling the intra-sire, intra-herd regression of offspring on dam. The daughter-dam regressions for milk and milk fat production were corrected to a single record basis according to the method described by Lush and Straus (7), to facilitate their comparison with those reported in other studies.

Phenotypic correlations were converted to a single record basis by the following formula:

$$\sqrt{\frac{1+(n-1)\,r}{n}}$$

n = number of production records.
r = repeatability of records (0.4 was used in this study).

Genetic correlations were estimated from the following variance and covariance formula:

$$r_{g_T g_P} = \frac{\frac{1}{2}(\Sigma TP' + \Sigma PT')}{\sqrt{(\Sigma TT')(\Sigma PP')}}$$

where T and P refer to type and production of the daughter and T' and P' refer to type and production of the dam.

The variances of the genetic correlations were computed by the method described by Reeve (10).

RESULTS AND DISCUSSION

Correlations. Intra-sire-intra-herd phenotypic and genetic correlations between production of milk and fat and type classification ratings (final and breakdown) are shown in Tables 2, 3, 4, and 5. The phenotypic correlations between the type ratings and production were generally low (.02 to .25). The differences in the phenotypic correlations among the three stratified groups and

TABLE 2

Phenotypic [a] and genetic correlations [b] between milk and milk fat production and type ratings of cows in low-producing herds

	Milk	Fat	Final	GA	DC	BC	MS	F & L	Rump
Milk		.62	.13	.09	.23	.08	.11	.02	.04
Fat	.76		.14	.11	.22	.10	.12	.02	.06
Final	.08	.09		.79	.38	.46	.80	.43	.50
GA	.01	.04	.99		.31	.48	.56	.47	.60
DC	.61	.63	.79	.75		.22	.28	.11	.21
BC	−.06	−.01	.65	.66	.26		.28	.23	.32
MS	.11	.15	.97	.90	.69	.05		.30	.33
F & L	−.17	−.18	.52	.53	−.07	.44	.46		.29
Rump	−.05	−.03	.76	.78	.35	.51	.60	.37	

Phenotypic correlations
Genetic correlations

[a] Phenotypic correlations are shown above and to the right of the diagonal line. The standard errors range from .01 to .02.

[b] Genetic correlations are shown below and to the left of the diagonal line. The standard error is approximately .10.

TABLE 3

Phenotypic [a] and genetic correlations [b] between milk and milk fat production and type ratings of cows in medium-producing herds

	Milk	Fat	Final	GA	DC	BC	MS	F & L	Rump
Milk		.62	.16	.12	.25	.10	.13	.06	.04
Fat	.80		.16	.12	.25	.10	.13	.05	.05
Final	.28	.25		.78	.39	.48	.81	.43	.50
GA	.02	.03	.97		.31	.50	.56	.50	.58
DC	.82	.84	.87	.65		.19	.32	.10	.16
BC	.33	.31	.73	.76	.37		.30	.25	.31
MS	.23	.17	.89	.74	.64	.55		.32	.33
F & L	.26	−.07	.68	.80	.25	.65	.40		.31
Rump	.12	.08	.72	.92	.27	.35	.41	.83	

Phenotypic correlations
Genetic correlations

[a] Phenotypic correlations are shown above and to the right of the diagonal line. The standard errors range from .01 to .02.

[b] Genetic correlations are shown below and to the left of the diagonal line. The standard error is approximately .10.

TABLE 4

Phenotypic [a] and genetic correlations [b] between milk and milk fat production and type ratings of cows in high-producing herds

	Milk	Fat	Final	GA	DC	BC	MS	F & L	Rump
Milk		.62	.14	.09	.24	.06	.11	.03	.04
Fat	.72		.16	.11	.24	.07	.13	.25	.05
Final	−.04	.02		.77	.37	.46	.78	.44	.49
GA	−.02	.02	.96		.24	.46	.53	.47	.58
DC	.61	.36	.33	.31		.18	.29	.09	.16
BC	.12	.19	.77	.85	.55		.28	.23	.29
MS	−.13	−.08	.89	.72	.07	.39		.32	.31
F & L	.07	−.01	.44	.64	−.19	.32	.13		.27
Rump	−.01	.07	.65	.70	.42	.55	.50	.19	

Phenotypic correlations
Genetic correlations

[a] Phenotypic correlations are shown above and to the right of the diagonal line. The standard errors range from .01 to .02.

[b] Genetic correlations are shown below and to the left of the diagonal line. The standard error is approximately .10.

TABLE 5

Phenotypic [a] and genetic correlations [b] between milk and milk fat production and type ratings of cows in Analysis I

	Milk	Fat	Final	GA	DC	BC	MS	F & L	Rump
Milk		.54	.16	.15	.20	.12	.13	.06	.08
Fat	.61		.17	.16	.20	.12	.14	.04	.08
Final	−.08	−.10		.82	.46	.49	.81	.42	.53
GA	−.04	−.07	.86		.42	.48	.63	.43	.59
DC	.04	.07	.96	1.00		.29	.38	.12	.24
BC	.01	−.24	.44	.72	.45		.34	.21	.31
MS	−.03	−.05	.85	.61	.36	−.15		.31	.35
F & L	−.07	−.08	.31	−.01	−.24	.01	.30		.29
Rump	.18	.07	.72	.85	.37	.46	.28	.37	

Phenotypic correlations
Genetic correlations

[a] Phenotypic correlations are shown above and to the right of the diagonal line. The standard errors range from .01 to .02.

[b] Genetic correlations are shown below and to the left of the diagonal line. The standard error is approximately .10.

Analysis I were small. The highest phenotypic and genetic correlations were between milk and fat production and between the production traits and dairy character. There was a high and similar degree of genetic correlation between dairy character and milk production in low-, average-, and high-producing herds. However, the genetic correlation between dairy character and fat production was smaller in the high-producing herds (.36) than in the low- (.63) and medium- (.84) herds. The genetic correlations between dairy character and milk and fat in Analysis I were not significant.

Phenotypic and genetic correlations between final type ratings and milk and fat production reported by other workers have been consistently low (3, 5, 11-14, 17). The results calculated in this study are in accord with previous findings. Freeman and Dunbar (2) reported a genetic correlation of −0.52. Their estimate, although quite different from the values in the present study, as well as those reported by other workers, had a large sampling error due to the small number of animals used.

The phenotypic correlations between the different type ratings showed little variation among the three stratified groups and Analysis I. In contrast, the genetic correlations varied more than the phenotypic correlation among the four groups. In all groups final type ratings showed uniformly high (> 0.75) phenotypic and genetic correlations with the subratings, general appearance, and mammary system. The corresponding correlations between final rating and the other four subratings ranged from 0.39 to 0.53 (phenotypic) and from 0.31 to 0.96 (genetic). These results are not surprising, because general appearance

and mammary system are given more emphasis than dairy character and body capacity in attaining the final rating. Discrepancies are apparent after comparing phenotypic correlations with comparable genetic correlations. These differences would indicate that the environmentally caused portion of the phenotypic correlation was usually different from the genetic part. Examples of this are the correlations between milk and milk fat production and dairy character, since the phenotypic correlations between milk and dairy character and milk fat and dairy character ranged from +0.20 to +0.25. However, in the stratified groups the estimates of genetic correlation ranged between 0.61 and 0.82, except in the high herds, where the genetic correlation between fat production and dairy character was 0.36.

Heritabilities. Intra-sire-intra-herd estimates of heritability of milk and fat production in this study were from 0.12 to 0.24, with standard errors of ±0.04 (Table 6). The level of herd production had no significant effect on the different

TABLE 6

Heritability [a] estimates based on intra-herd, intra-sire regressions of daughter on dam

	Low group	Medium group	High group	Analysis I
Milk	.20	.24	.19	.14
Milk fat	.19	.21	.17	.12
Final rating	.24	.20	.18	.15
Gen. appearance	.24	.19	.21	.12
Dairy character	.09	.09	.06 [b]	.03 [b]
Body capacity	.15	.12	.14	.10
Mammary system	.21	.18	.14	.12
Feet and legs	.18	.16	.12	.12
Rump	.31	.27	.24	.31

[a] The standard errors ranged from .03 to .04.
[b] Not significant ($P > 0.05$).

estimates. The estimates for the Analysis I group were 0.12 and 0.14, which were significantly lower than the values for the medium group. The estimates in this study agree with those reported in previous studies on H.I.R. and D.H.I.A. data (1, 3, 4, 6-8, 12-14), but are lower than the figure of 0.36 reported by Laben and Herman (5) for milk production.

The heritability estimates for type ratings in the present study also were similar to those reported by other workers (3, 11, 12-14) for Jersey and Holstein-Friesian cattle. The heritabilities of final type ratings for Ayrshire cattle (2 and 16) and for Brown Swiss (4) were considerably larger than those reported for Jersey and Holstein cattle.

The amount of genetic improvement in production that could be obtained by selection on type alone can be estimated from the heritability coefficients and genetic correlations. The relative genetic improvement in milk production when selection is based on a type rating, in contrast to selection based on one milk record is:

$$\sqrt{\frac{\text{heritability type}}{\text{heritability milk yield}}} \times \text{genetic correlation between type and milk yield}$$

The relative degrees of progress expected in milk production by selection on either final or dairy character ratings, as contrasted with selection on production alone are:

Production group	Final rating	Dairy character rating
Low	.09	.41
Medium	.26	.50
High	.04	.34

In each of the stratified herd groups the relative progress to be expected in production is in favor of the dairy character rating over final type rating. However, neither is expected to be as effective as production records in selecting for production. For those breeders interested in improving both type and production traits, some attention to both characteristics will be required. In addition, genetic improvement in both traits will be slower than if only one trait was considered in the selection program.

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USE OF PART LACTATION RECORDS IN SIRE EVALUATION

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SUMMARY

Estimates of sire and error components of variance of monthly records, and functions of monthly records and sire components of covariance between functions of monthly records and 10-mo. yield, obtained from the paternal half-sib analysis of monthly milk records of 1,526 Holstein cows sired by 145 sires used in artificial insemination, are used to determine the number of part records required to predict the breeding value of a sire for 10-mo. yield with an accuracy equal to prediction on the basis of a fixed number of 10-mo. records. The estimates of variance components are presented for monthly records, cumulative monthly records, linear functions of sequential monthly records, and cumulative and sequential functions of bimonthly and trimonthly records. The estimates of sire covariance components with 10-mo. yield also are given. The required number of part records of these functions to be as accurate in predicting the breeding value of a bull for 10-mo. milk yield as 20, 30, 40, 50, 100, 150, and 200 10-mo. records are listed in tabular form. Corresponding numbers of fifth test-day records required for equal accuracy are 24, 35, 47, 58, 111, 160, and 205. Only slightly more 7- or 8-mo. records than 10-mo. records are needed for equal accuracy of prediction.

Part lactation records can be useful in sire evaluation. The consequence of using part records is a recurring question. Several investigators, Searle (6) and Madden *et al.* (2), have suggested that part records may be used in selection programs for improving genetic merit for complete lactation yield. These suggestions have been based on the high genetic correlations which exist between portions of records and complete records. There is a question of how many part records of a sire's daughters are needed to estimate a sire's breeding value for complete lactation milk yield as accurately as a fixed number of complete records. This paper presents a method for determining the number of part records required. Analysis of actual data is then used to determine the number required for various functions of part records when the accuracy of prediction is fixed.

DATA AND ANALYSIS PROCEDURE

The DHIA milk records were from 1,526 Holstein cows sired by 145 AI (artificial insemination) sires. Ten monthly test-day records were available for each lactation, beginning with the 1st mo. of lactation and ending with the 10th mo. The sum of the ten test-day records which made up a cow's record was defined to be a complete lactation record. The sample of monthly test records was obtained from November, 1957, through December, 1959. The monthly records were adjusted for age at calving and season of calving by correction factors described by VanVleck and Henderson (7). The corrected records expressed as deviations from herd averages were analyzed according to a within- and between-sires analysis. The sire and error components of variance

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and covariance were estimated by the procedure described by VanVleck and Henderson (9).

THEORETICAL CONSIDERATIONS

Let our model be:

$P_{ij} = \mu_p + s_i + e_{ij}$ and

$C_{ij} = \mu_c + g_i + f_{ij}$, where

P_{ij} is the part record of the j^{th} daughter of the i^{th} sire,

C_{ij} is the complete record of the j^{th} daughter of the i^{th} sire,

μ_p and μ_c are fixed population parameters,

s_i is the part record effect associated with the i^{th} sire,

g_i is the complete record effect associated with the i^{th} sire,

e_{ij} is the random error effect associated with the part record of the j^{th} daughter of the i^{th} sire, and

f_{ij} is the random error effect associated with the complete record of the j^{th} daughter of the i^{th} sire.

It is assumed that the s_i, g_i, e_{ij}, and f_{ij} are normally and independently distributed with zero means and variances σ_s^2, σ_g^2, σ_e^2, and σ_f^2, respectively; that the covariance between s_i and g_i is σ_{sg}, between e_{ij} and f_{ij} is σ_{ef}; and that all other covariances are zero. This model assumes correction has been made for all effects other than those due to sire and random effects.

The means of the part and complete records made by daughters of the i^{th} sire in terms of the model are

$$\overline{P}_{i.} = s_i + \frac{e_{i.}}{n_i} \text{ and}$$

$$\overline{C}_{i.} = g_i + \frac{f_{i}}{n_i'} \text{ where}$$

n_i daughters have part records, n_i' daughters have complete records, and the usual dot notation indicates summation over that subscript. The variances of these means and the covariances between them are

$$V(\overline{P}_{i.}) = \sigma_s^2 + \frac{\sigma_e^2}{n_i},\ V(\overline{C}_{i.}) = \sigma_g^2 + \frac{\sigma_f^2}{n_i'}, \text{ and Cov } (\overline{P}_{i.}, \overline{C}_{i.}) = \sigma_{sg} + \frac{\sigma_{ef}}{n_i'}$$

when $n_i' > n_i$ and all n_i part records are also included in the n_i' complete records.

The correlation between the mean of n_i part records and mean of n_i' complete records by daughters of the same sire is:

$$r_{P(n),C(n')} = \frac{\text{Cov}(\overline{P}_{i.}\ \overline{C}_{i.})}{[V(\overline{P}_{i.})V(\overline{C}_{i.})]^{1/2}} = \frac{\sigma_{sg} + \frac{\sigma_{ef}}{n_i'}}{[(\sigma_s^2 + \frac{\sigma_e^2}{n_i})(\sigma_g^2 + \frac{\sigma_f^2}{n_i'})]^{1/2}}$$

again when $n_i' > n_i$ and all n_i part records are also included in the n_i' complete records.

If n_i' becomes very large, the correlation between the mean of n_i part records and the mean of all possible complete records by daughters of the same sire is obtained. This correlation or accuracy of prediction is the same as that between the mean of n_i part records and the sire's breeding value for complete lactation yield, which may be written as:

$$\underset{n' \longrightarrow \infty}{{}^{r}P(n), C(n')} \longrightarrow \frac{\sigma_{sg}}{[(\sigma_s^2 + \frac{\sigma_e^2}{n_i})(\sigma_g^2)]^{1/2}}$$

The accuracy of predicting complete record performance of all possible daughters of the i^{th} sire given a sample of n_i' daughters is $\frac{\sigma_g}{[\sigma_g^2 + \frac{\sigma_f^2}{n_i'}]^{1/2}}$ which is the correlation between the mean of a sample of n_i' complete records and the sire's breeding value for total yield. The number, n_i, of part records of daughters of a sire needed to predict future complete records with the same accuracy as n_i' complete records can be obtained by equating the two correlations and solving for n_i when n_i' is fixed:

$$\frac{\sigma_{sg}}{[(\sigma_s^2 + \frac{\sigma_e^2}{n_i})\sigma_g^2]^{1/2}} = \frac{\sigma_g}{[\sigma_g^2 + \frac{\sigma_f^2}{n_i'}]^{1/2}}.$$

To simplify the expression, set $\frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_f^2}{n_i'}}$ equal to A.

Then $n_i = \frac{\sigma_e^2 \sigma_g^2 A}{(\sigma_{sg})^2 - \sigma_g^2 \sigma_s^2 A}$. An acceptable solution can be obtained only if the denominator is greater than zero. If the denominator becomes very small (approaches zero), then the number required becomes very large (approaches an infinite number). Searle (5) has developed by a different approach an equivalent procedure for determining the required number of part records which is based on known heritabilities and genetic correlations.

RESULTS AND DISCUSSION

The sire and error components of variance were estimated for monthly records, cumulative monthly records, the linear functions of sequential monthly records described by VanVleck and Henderson (8), cumulative bimonthly and trimonthly records, and linear functions of bimonthly and trimonthly records. The sire components of covariance between these functions of monthly records

TABLE 1

Estimates of variance and covariance components of monthly records [a]

	Month									
	1	2	3	4	5	6	7	8	9	10
$\hat{\sigma}_s^2$	2.39	5.42	7.35	5.12	4.15	3.11	2.50	2.77	2.59	2.53
$\hat{\sigma}_e^2$	88.16	122.83	124.50	102.81	85.23	77.80	71.08	74.63	87.38	120.54
$\hat{\sigma}_{s,\,s_T}$	24.06	32.17	44.79	37.70	35.88	30.38	27.47	25.69	26.40	19.66

[a] $\hat{\sigma}_s^2$ is the estimated sire component of variance.

$\hat{\sigma}_e^2$ is the estimated error component of variance.

$\hat{\sigma}_{s,\,s_T}$ is the estimated sire component of covariance between the 10-mo. record and the monthly record.

TABLE 2

Estimates of variance and covariance components of cumulative monthly records

	Cumulative month								
	2	3	4	5	6	7	8	9	10
$\hat{\sigma}_s^2$	15.05	41.86	74.54	112.31	146.39	184.08	222.00	266.34	305.80
$\hat{\sigma}_e^2$	334.46	767.84	1,306.41	1,886.00	2,514.72	3,154.73	3,805.10	4,410.21	5,095.40
$\hat{\sigma}_{s,\,s_T}$	56.22	101.02	138.72	174.58	204.98	232.47	258.17	284.56	305.80

TABLE 3

Estimates of variance and covariance components of linear functions of sequential monthly records [a]

	Sequential month								
	2	3	4	5	6	7	8	9	10
$\hat{\sigma}_s^2$	145.23	221.06	238.54	268.66	259.30	267.54	277.94	256.30	305.80
$\hat{\sigma}_e^2$	3,124.80	3,766.68	4,150.73	4,469.97	4,636.78	4,726.09	4,899.69	4,256.54	5,095.40
$\hat{\sigma}_{s,\,s_T}$	170.22	239.12	254.67	277.87	280.44	284.00	290.89	279.00	305.80

[a] The functions of sequential monthly records are those described by VanVleck and Henderson (8).

TABLE 4

Estimates of variance and covariance components of cumulative and of linear functions of bimonthly and trimonthly records [a]

	Sequential function					Cumulative				
	Bimonthly set		Trimonthly set			Bimonthly set		Trimonthly set		
	1	2	1	2	3	1	2	1	2	3
$\hat{\sigma}_s^2$	323.38	256.81	246.26	236.70	301.45	82.94	70.07	27.76	28.81	33.97
$\hat{\sigma}_e^2$	4,889.21	5,024.91	4,478.52	4,754.79	4,747.78	1,262.13	1,391.58	501.69	573.39	534.54
$\hat{\sigma}_{s,\,s_T}$	313.58	279.85	267.92	270.60	302.81	158.61	145.64	89.23	93.75	101.57

[a] The bimonthly and trimonthly sets are those described by VanVleck and Henderson (8).

and 10-mo. yield were also estimated. These variance and covariance estimates are given in Table 1 for monthly records, Table 2 for cumulative monthly records, Table 3 for linear functions of sequential monthly records, and Table 4 for cumulative bimonthly and trimonthly records and for linear functions of bimonthly and trimonthly records.

The number of functions of part records needed to give the same accuracy for predicting genetic merit for complete yield as 20, 30, 40, 50, 100, 150, and 200 complete 10-mo. records were obtained by substituting the appropriate constants into the equation for determining n_i, the number of part records. The values for accuracy of prediction when using 20, 30, 40, 50, 100, 150, and 200 complete records are 0.74, 0.80, 0.84, 0.87, 0.93, 0.95, and 0.96, respectively. The accuracy, $A^{1/2}$, can be estimated by substituting the sire and error components into the equation

$$A^{1/2} = \left(\frac{\hat{\sigma}_s^2}{\hat{\sigma}_s^2 + \frac{\hat{\sigma}_e^2}{n_i'}} \right)^{1/2}$$

The tenth cumulative month of Table 2 corresponds to complete lactation yield. From Table 2 are obtained the values, $\hat{\sigma}_s^2 = 305.80$ and $\hat{\sigma}_e^2 = 5095.40$, for complete lactation production, which can be substituted into the equation for $A^{1/2}$.

The number of part records required to be as accurate as 20, 30, 40, 50, 100, 150, and 200 10-mo. records in predicting a sire's breeding value for 10-mo. milk yield are given in Table 5 for monthly records, Table 6 for cumulative monthly records, Table 7 for linear functions of sequential monthly records, and Table 8 for sequential functions of bimonthly or trimonthly records and for cumulative bimonthly or trimonthly records. Sire proofs based on the fifth monthly test-day record require only a few additional records to be as accurate as proofs based on 10-mo. records. If this procedure were followed, a gain of 5 mo. could be made in deciding whether to use or sell a tested sire. The saving in costs may be substantial or the reduced time of proving may be used to test

TABLE 5

Number of single month of lactation records needed to be as accurate as 20, 30, 40, 50, 100, 150, and 200 complete records [a]

No. of complete records	Month of lactation									
	1	2	3	4	5	6	7	8	9	10
20	82	157	27	30	24	32	35	63	55	~
30	159	~	44	49	35	49	53	126	91	~
40	302	~	64	70	47	67	71	256	136	~
50	659	~	90	95	58	85	90	665	194	~
100	~	~	406	339	111	190	186	~	1,233	~
150	~	~	~	2,274	160	323	289	~	~	~
200	~	~	~	~	205	496	401	~	~	~

[a] ~ indicates that the denominator of the formula is negative.

TABLE 6

Number of cumulative monthly records required to be as accurate as 20, 30, 40, 50, 100, 150, and 200 complete records

No. of complete records	Cumulative months of lactation									
	1	2	3	4	5	6	7	8	9	10
20	82	86	40	32	27	24	23	21	20	20
30	159	325	76	56	44	37	35	33	30	30
40	302	~	142	89	65	52	48	44	41	40
50	659	~	292	140	92	68	61	55	51	50
100	~	~	~	~	476	181	143	118	104	100
150	~	~	~	~	~	401	257	189	158	150
200	~	~	~	~	~	1,027	428	270	215	200

TABLE 7

Number of sequential monthly records required to be as accurate as 20, 30, 40, 50, 100, 150, and 200 complete records [a]

No. of complete records	Sequential months of lactation									
	1	2	3	4	5	6	7	8	9	10
20	82	110	31	28	23	22	22	21	20	20
30	159	146	54	45	36	33	33	32	30	30
40	302	~	86	67	50	44	45	43	41	40
50	659	~	133	94	66	55	56	54	51	50
100	~	~	~	467	173	114	118	109	105	100
150	~	~	~	~	376	175	185	166	160	150
200	~	~	~	~	919	240	260	224	219	200

[a] The functions of sequential monthly records are given by VanVleck and Henderson (8).

TABLE 8

Number of sequential and cumulative bimonthly and trimonthly records required to be as accurate as 20, 30, 40, 50, 100, 150, and 200 complete records [a]

No. of complete records	Sequential function					Cumulative				
	Bimonthly set		Trimonthly set			Bimonthly set		Trimonthly set		
	1	2	1	2	3	1	2	1	2	3
20	18	24	24	24	19	19	24	25	24	19
30	28	36	38	35	29	28	37	39	36	29
40	37	47	52	46	39	38	49	55	48	39
50	46	60	67	58	48	47	62	72	60	49
100	94	120	162	111	98	97	128	192	121	99
150	144	181	308	162	150	149	199	429	184	152
200	196	244	559	209	203	204	275	1,122	247	207

[a] Bimonthly and trimonthly sets are those described by VanVleck and Henderson (8).

more bulls with no greater expenditure. Since sires with preliminary evaluations based on relatively small numbers of daughters are always subjected to re-examination when more records become available, 5th-mo. records appear suitable for preliminary evaluation of sires. Sires with extremely poor 5th-mo. records could be rejected immediately, sires with average 5th-mo. records could be re-evaluated on the basis of complete records, and exceptional sires could be put back into service. Preliminary evaluation on functions of the first five or six monthly records or on cumulative production of the first 5 or 6 mo. also seems warranted.

It appears unnecessary to use more than 7- or 8-mo. cumulative data for the evaluation of sires. The numbers required for equal accuracy of proof are only slightly larger for 8-mo. records than for 10-mo. records. The 2- or 3-mo. time advantage heavily outweighs the slightly increased accuracy of prediction gained from waiting for 10-mo. records.

Fewer sets of bimonthly records are needed for estimating breeding value for total lactation yield than numbers of 10-mo. records when the bimonthly testing begins in the 1st mo. of lactation. Slightly more bimonthly records are needed when testing begins in the 2nd mo. of lactation. Similarly, trimonthly test results are nearly as accurate in predicting a sire's true breeding value for 10-mo. yield as complete records. These results are due to the high genetic relationship between bimonthly test yields and 10-mo. yield and between trimonthly test yields and 10-mo. yield.

The reviewer has appropriately pointed out that the question as to what effect the selection on only a few months of the first records would be expected to have on lifetime production if all sire selections were pushed to this extreme is certain to be raised, since when 305-day first records are used in sire evaluation this point is the subject of considerable controversy. All available evidence, however, indicates that selection on first records does not influence the increase of yield in later lactations. Robertson and Khishin (4) concluded on the basis of regressions of near zero for increase from first lactation yield to second and second to third lactation yield on first lactation contemporary comparison that, "selection on first lactation yield should not, therefore, change the increase of yield with age. . . ." An earlier study, Hickman and Henderson (1), also does not support the contention that selection based on first records will reduce the increase of production with age.

Lifetime production would seem nearly synonymous with longevity. Parker *et al.* (3) concluded from an analysis of Beltsville data collected during a 40-yr. period that heritability of longevity is low if not zero, that there is no evidence of association between longevity and the first lactation, and that there is a slight tendency for higher producers to remain in the herd longer, even in the absence of selection for production. It would seem, then, on the basis of somewhat indirect evidence, that selection on first records will not adversely affect lifetime production.

The high genetic correlations between part records and complete records, Madden *et al.* (2), Searle (6), and VanVleck and Henderson (9), imply that

the above argument can be successfully transferred to the influence of selection on part records on lifetime production, provided that the genetic correlations of the part records used with complete records are near unity, as is the case with 5-mo. or longer records.

CONCLUSIONS

Sire selection on the basis of 7- or 8-mo. yield rather than on the basis of 10-mo. yield is indicated. The time advantage appears to overbalance the slight increase of accuracy of selection on total yield.

Preliminary evaluation of AI sires based on the fifth monthly test or on a function of 5-mo. records appears important as a first stage of selection. Below-average sires could be rejected on this amount of information. Questionable decisions could be made on the basis of more complete (7- or 8-mo.) records. Outstanding sires could be put into heavy service 5 mo. earlier than if evaluation were made on 10-mo. records.

Bimonthly testing appears to be a practical procedure from the position of sire evaluation. Approximately the same number of sets of bimonthly records as 10-mo. records are required to prove a bull with equal accuracy for prediction of breeding value for 10-mo. yield.

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EXTENDING PART LACTATION MILK RECORDS BY REGRESSION IGNORING HERD EFFECTS

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SUMMARY

Regression coefficients for predicting 10-mo. milk yield from single test-day records, from cumulative test-day records of less than 10 mo. duration, from bimonthly and trimonthly test-day records, and from other combinations of test-day records are estimated ignoring herd effects. The records, adjusted for age at calving and season of calving, used for estimating these coefficients were from 9,036 Holstein cows in five New York counties. These regression coefficients are compared with intraherd coefficients reported earlier which were estimated from the same data. The relative efficiencies of the two procedures are compared to determine which procedure is more practical. For most combinations of records regression ignoring herd effects is less than 20% less efficient and in many cases less than 10% less efficient than regression considering herd effects. It is concluded that prediction of 10-mo. milk yield by regression ignoring herd effects is more practical for most situations, because of computational advantages although the accuracy of prediction is slightly less in all situations than for intra-herd prediction.

Many reports (3, 4, 6, 8, 9, 13, 14, and 15) have shown relatively high correlations between milk yields of part lactations of varying lengths and complete lactation yield. Most of these reports have been of total correlations which were obtained without considering herd or other effects. Recently, Madden *et al.* (10), also ignoring herd effects, have reported regression coefficients for extending monthly test day records and cumulative monthly test-day records to a complete yield basis. These regressions ignored herd effects but were calculated separately for less than 3 yr. of age at calving and more than 3 yr. of age at calving. Fritz *et al.* (5), also using Michigan D.H.I.A. records, concluded that herd effects on regression factors for extending part records were not important. This conclusion was based on an analysis of variance of regression coefficients. VanVleck and Henderson (17) reported within herd regressions of complete lactation milk yield on various combinations of age and season adjusted monthly test-day records. All such reports either considering herd effects or ignoring herd effects have emphasized the relatively large correlations between part lactation yields for 5 or more mo. and 10-mo. lactation yield.

The accuracy of prediction on a within-herd basis (intra-herd regression) is greater than of prediction ignoring herd effects (total regression), but the difficulty of using within-herd regression coefficients is considerable for commercial or cooperative dairy records processing centers. In order to use intra-herd regression coefficients in prediction equations herd averages for total yield and for part lactation yield are necessary. These averages can not be obtained economically in many cases. The herd means are also likely to be estimated with considerable error. Therefore, regression equations ignoring herd effects would be desirable in order to facilitate economical extension of part records. Estimates of population averages would be used in the prediction equations rather than herd averages. The former

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would be easy to obtain and would be subject to less error than would estimates of herd means. Such prediction equations, however, must be reasonably accurate. The purpose of this paper is to present regression coefficients which may be used to estimate lactation milk yield from various functions of monthly test-day records ignoring herd effects and to compare the efficiency of these predictors with comparable predictors obtained from regression on a within-herd basis.

DATA

The records used in this study were the same as those used by VanVleck and Henderson (17) for the estimation of within-herd regression coefficients. The D.H.I.A. milk records were made by 9,036 Holstein cows in five New York counties. Ten monthly test-day records were available for each cow's lactation beginning with the first month of lactation and ending with the tenth. Zero production was defined to be a valid test-day yield. The first of the test-day records had to be taken less than 50 days after calving. Otherwise, the lactation record was discarded as were lactations having missing test-day records. The sum of the first ten test-day records was defined to be a complete yield. Multiplication by 30.5 estimates the 305-day record. The monthly records were adjusted for age at calving and season of calving with ratio factors described by VanVleck and Henderson (16).

ANALYSIS PROCEDURE

The usual normal equations were solved to estimate the desired regression coefficients. The right- and left-hand sides of the normal equations were made up of total sums of squares and cross-products corrected for means. The reduction in total sum of squares of total yield due to regression (the multiple or simple correlation coefficient squared, R^2) was computed for each regression function. Functions were estimated for the same combinations of test-day records as those treated by Van Vleck and Henderson (17) on a within-herd basis.

Clearly, a method is needed to determine the relative effectiveness of the two regression procedures (within-herd versus ignoring herds). Comparison of the multiple or simple R's does not adequately show the relative efficiency of the two procedures. The R^2 associated with within-herd regression describes the amount of variation in within-herd variance which is accounted for by within-herd regression, while the R^2 associated with regression ignoring herds is the amount of the total variance (corrected for the mean) which is accounted for by regression ignoring herd effects. Certainly, these R's can not be compared. What is of concern is the variance of the difference between actual and predicted yield.

The residual variance not accounted for by total regression is $(1 - R_T^2)\hat{\sigma}_y^2$, where R_T^2 is the square of the multiple or simple correlation coefficient when herd effects are ignored and $\hat{\sigma}_y^2$ is the estimated variance of total yield, $\dfrac{\sum_{i=1}^{N} Y_i^2 - \dfrac{(\sum_{i=1}^{N} Y_i)^2}{N}}{N-1}$.

On the other hand, the variance not accounted for by within-herd regression is

$(1-R_w^2)\,\hat{\sigma}_e^2$, where R_w^2 is the square of the correlation coefficient on a within-herd basis and $\hat{\sigma}_e^2$ is the estimated error variance of total yield after eliminating herd variance, $\dfrac{\sum_{j=1}^{s}\sum_{k=1}^{n_j} Y_{jk}^2 - \sum_{j=1}^{s}\dfrac{(\sum_{k=1}^{n_j} Y_{jk})^2}{n_j}}{N-s}$. Note that $\sum_{j=1}^{s}\sum_{k=1}^{n_j} Y_{jk}^2 = \sum_{i=1}^{N} Y_i^2$, $\sum_{j=1}^{s} n_j = N =$ the total number of observations, and $s =$ the number of herds. These two values are the residual variances of prediction for the two procedures. The prediction procedure which provides the smaller error variance is defined to be the better of the two procedures. Experience leads to the belief that $(1-R_w^2)\hat{\sigma}_e^2$ is usually smaller than $(1-R_1^2)\hat{\sigma}_y^2$. Then the relative efficiency of the two procedures as a per cent can be defined as $E = 100\left(\dfrac{V_1}{V_2}\right) = 100\left[\dfrac{(1-R_1^2)\hat{\sigma}_y^2}{(1-R_w^2)\hat{\sigma}_e^2}\right]$. The absolute errors, of course, are $\sqrt{V_1}$ and $\sqrt{V_2}$, which correspond to the standard deviations of the difference between actual and predicted records for the two procedures.

Even with a measure of relative efficiency defined there remains another difficulty. Suppose Method 2 is 20% more efficient than Method 1. Should Method 1 be used rather than Method 2? This problem, naturally enough, is not amenable to an easy solution. The experimenter or the user of the procedures must make the final decision. He will be guided by the importance of the additional error and by the importance of the relative difficulties of computation of the two procedures. Perhaps, in the present situation, the ease of computation of Procedure 1 would more than counterbalance an increased error variance of 20% associated with Procedure 1. Then the using agency would choose Procedure 1. Or, if the user decided that the ease of Procedure 1 is not as important as the increased inaccuracy, then Procedure 2 would be used.

RESULTS AND DISCUSSION

Regression coefficients. The means of the monthly test-day milk records and the means of cumulative monthly test records are shown in Table 1. These values are comparable to those reported by Madden *et al.* (10) for Michigan data for cows 3 yr. and older at calving, except that the New York means drop off more rapidly toward the end of the lactation period. Estimates of average monthly milk production can be obtained by multiplying the test-day means by 30.5.

Regression factors ignoring herd levels for predicting total yield from a single monthly test-day record are given in Table 2. These values may be compared with the comparable within-herd regression coefficients given in Table 2 of VanVleck and Henderson (17). For each monthly test-day period the regression coefficient, b, is larger when herd effects are ignored. The b's ignoring herds are larger than the within-herd b's. A not very precise test of significance of the difference can be accomplished. The standard error of a b is about 0.06 which makes the variance about 0.0036. The variance of $(b_i - b_j)$ is less than 2(0.0036) since any covariance between b_i, total regression, and b_j, intra-herd regression, in our problem is ex-

TABLE 1

Means of age and season adjusted monthly test-day records and means of cumulative monthly test-day records

	Month									
	1	2	3	4	5	6	7	8	9	10
Single months	57.1	59.8	56.9	51.7	46.7	41.5	35.2	28.3	18.9	11.8
Cumulative months	57.1	116.9	173.8	225.5	272.2	313.7	348.9	377.2	396.1	407.9

TABLE 2

Regression factors for estimating total lactation yield from a single monthly test record [a]

	Month of lactation									
	1	2	3	4	5	6	7	8	9	10
b	5.52	5.59	5.86	6.58	7.14	7.51	7.83	7.33	6.08	4.33
s_b	0.06	0.04	0.04	0.04	0.04	0.04	0.05	0.06	0.07	0.07
R	0.67	0.82	0.86	0.89	0.90	0.89	0.87	0.81	0.68	0.52

[a] b is the regression coefficient, s_b is the standard error of the regression coefficient, and R is the correlation coefficient between the predicted and actual value.

TABLE 3

Regression factors for estimating total lactation yield from sequential test-day data

Sequential months	Monthly regression coefficients										R[a]
	1	2	3	4	5	6	7	8	9	10	
1	5.52	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.67
2	1.68	4.57	.....	.....	.....	.....	.....	.....	.....	.....	0.84
3	1.32	1.46	3.86	.....	.....	.....	.....	.....	.....	.....	0.89
4	1.04	0.95	1.35	3.86	.....	.....	.....	.....	.....	.....	0.92
5	0.77	0.90	1.09	1.52	3.51	.....	.....	.....	.....	.....	0.95
6	0.74	0.89	1.07	1.11	1.36	3.11	.....	.....	.....	.....	0.96
7	0.83	0.98	0.97	0.97	1.06	1.14	2.89	.....	.....	.....	0.97
8	0.88	1.07	1.01	0.91	0.90	0.98	0.89	2.68	.....	.....	0.99
9	0.95	1.03	1.04	0.96	0.97	0.98	1.00	0.95	1.90	.....	1.00
10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] R is the multiple correlation coefficient between the predicted and actual value.

TABLE 4

Regression factors for estimating total lactation yield from sequential monthly test-day records when first months are missing

Sequential months	Monthly regression coefficients										R
	1	2	3	4	5	6	7	8	9	10	
1	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.33	0.52
2	.....	.....	.....	.....	.....	.....	.....	.....	6.08	−0.11	0.68
3	.....	.....	.....	.....	.....	.....	.....	6.87	0.15	0.55	0.82
4	.....	.....	.....	.....	.....	.....	5.80	1.61	0.59	0.76	0.89
5	.....	.....	.....	.....	.....	4.60	1.79	1.14	0.68	0.91	0.93
6	.....	.....	.....	.....	3.68	1.72	1.26	0.86	0.81	0.99	0.96
7	.....	.....	.....	3.00	1.47	1.22	0.99	0.83	0.92	1.00	0.98
8	.....	.....	2.03	1.30	1.22	1.19	0.89	0.92	1.01	0.94	0.99
9	.....	1.47	1.03	1.03	1.13	1.08	0.95	1.02	1.00	0.94	1.00
10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

pected to be positive. Tabulated t-values can be compared with our sample t statistic, $\frac{b_i - b_j}{\sqrt{2(0.0036)}}$. The degrees of freedom associated with the test can only be approximated but are probably not very critical in the present situation because of the large number of degrees of freedom associated with the variances of b_i and b_j. Even though the total and intra-herd b's are significantly different, this result does not necessarily preclude the use of total regressions over all herds, as will be shown later.

The total correlations between single monthly test-day records and total yield are very similar to those presented by Madden *et al.* (10).

An example of the use of regression factors to estimate total yield from a single monthly test-day record follows: suppose the fourth test-day record is 61.7 lb. of milk. Then the predicted sum of ten test-day records is $\hat{y} = 407.9 + 6.58(61.7 - 51.7) = 473.7$. Multiplication by 30.5 gives the predicted 305-day yield, 14,448 lbs. of milk. The predicted sum is given in general by $\hat{y} = \hat{\mu}_y + b_i (X_i - \hat{\mu}_i)$ where $\hat{\mu}_y$ is the estimate of the population average sum of test-day records, b_i is the regression coefficient associated with the i^{th} month, and $\hat{\mu}_i$ is the estimate of the population average test-day record in the i^{th} month.

Multiple regression factors ignoring herd effects are given in Table 3 for the situation when the last monthly test-day records are lacking and in Table 4 for the situation when the first monthly records are missing. Relatively great accuracy of prediction can be obtained from a linear function of the first three or more monthly test-day records. The expected correlation with total yield when using the first three records is 0.89. The correlation increases with the addition of more monthly test records. The total multiple regression coefficients appear to be different from the intra-herd coefficients reported in Tables 3 and 4 of VanVleck and Henderson (17) unless five or more monthly records are included in the function.

The total regression factors for extending cumulative monthly test-day records are of more interest than the multiple factors, since records are maintained in a cumulative manner by most records-processing laboratories. These coefficients are reported in Table 5 for the situation when the last monthly records are missing, and in Table 6 when the first monthly records are not available. It is interesting to notice that the accuracy of prediction using cumulative records is only slightly less than using a linear function of the same monthly records. The corresponding intra-herd regression factors for cumulative months are given in Tables 5 and 6 of VanVleck and Henderson (17). The intra-herd and total regression coefficients are very similar for the cumulative records, especially when the last months are missing and the first four or more monthly test records are included in the cumulative sum.

Bimonthly or trimonthly testing is a possibility for reducing the cost of testing for herd owners. The high correlation between bimonthly and monthly testing reported by several researchers, (1, 2, 7, 11, and 12), advances this possibility. The within-herd prediction equations based on bimonthly and trimonthly testing presented in Tables 7 and 8 of VanVleck and Henderson (17) further suggest the practicality of such testing procedures. Total regression coefficients for linear func-

TABLE 5

Regression factors for estimating total lactation yield from cumulative test-day records

	Cumulative month of lactation								
	1	2	3	4	5	6	7	8	9
b	5.52	3.26	2.28	1.79	1.52	1.34	1.21	1.12	1.05
R	0.67	0.82	0.87	0.91	0.93	0.95	0.97	0.98	0.99

TABLE 6

Regression factors for estimating total lactation yield from cumulative test-day records when first-month records are missing

	Cumulative month of lactation								
	10	9	8	7	6	5	4	3	2
b	4.83	2.91	2.40	2.11	1.83	1.59	1.40	1.22	1.08
R	0.52	0.63	0.74	0.83	0.89	0.93	0.96	0.98	1.00

TABLE 7

Regression factors for estimating total lactation yield from sequential bimonthly and trimonthly test-day records

Monthly set	Month of lactation										R
	1	2	3	4	5	6	7	8	9	10	
1	1.32	.....	2.17	.....	1.94	.....	2.00	.....	2.36	.....	0.99
2	.....	1.98	.....	2.03	.....	2.15	.....	2.05	.....	1.32	0.99
1	1.58	.....	.....	3.17	.....	.....	4.15	.....	.....	.....	0.96
2	.....	2.57	.....	.....	2.87	.....	.....	3.51	.....	.....	0.98
3	.....	.....	3.20	.....	.....	3.34	.....	.....	2.59	.....	0.98

TABLE 8

Regression factors for estimating total lactation yield from cumulative bimonthly and trimonthly test-day records

	Bimonthly set		Trimonthly set		
	1	2	1	2	3
b	1.96	1.95	2.94	2.92	3.09
R	0.99	0.99	0.95	0.97	0.98

TABLE 9

Relative efficiency of total and intra-herd regression for predicting total lactation yield from single monthly test-day milk records

	Month									
	1	2	3	4	5	6	7	8	9	10
V_1[a]	4,585	2,828	2,252	1,802	1,736	1,828	2,145	2,998	4,784	6,446
V_2[b]	3,774	2,425	1,916	1,599	1,560	1,612	1,775	2,209	3,176	4,082
E[c]	121	117	118	113	111	113	121	136	151	158

[a] V_1 is the residual variance of the sum of the first ten test records (total yield) not accounted for by total regression. The regression coefficients are shown in Table 2.

[b] V_2 is the residual variance of total yield not accounted for by intra-herd regression. The intra-herd regression coefficients are shown in Table 2 of VanVleck and Henderson (17).

[c] E is the ratio $100\left(\frac{V_1}{V_2}\right)$, which is the relative efficiency of the two procedures.

tions of bimonthly and trimonthly test-day records are shown in Table 7 and coefficients for cumulative bimonthly and trimonthly test records in Table 8. The total regression coefficients do not appear different from the intraherd coefficients. Some are almost identical. Again, it is apparent that the linear functions of bimonthly or trimonthly test-day records are only slightly better predictors of total yield than the corresponding cumulative sums of bimonthly and trimonthly test records.

Comparison of the efficiency of the total regression and intra-herd regression equations. As stated previously, the residual variances not accounted for by regression can be used to compare the two procedures which are used to predict total yield. Procedure 1 is prediction based on regression ignoring herd effects and Procedure 2 is prediction based on within-herd regression. The relative efficiencies of the two procedures when using single test-day records are given in Table 9, together with the residual variances. The relative efficiencies are smallest for the fourth, fifth, and sixth months—the same months which provide the best estimates of total yield. The difference in variances is about 200 for these residuals, yet the differences in the average errors (the square roots of the residual variances) are only about 2 or 3 lb. of milk. For example, the square roots of the residual variances for the fourth month are: $(V_1)^{\frac{1}{2}} = (1802)^{\frac{1}{2}} = 42.5$ and $(V_2)^{\frac{1}{2}} = (1599)^{\frac{1}{2}} = 40.0$. This, then, shows an average error of measurement of 2.5 lb. more for Procedure 1 than Procedure 2. The average total yield from Table 1 is 407.9 lb. The percentage of additional error is thus less than 1% relative to total yield. It would appear that if prediction were based on the fourth, fifth, or sixth monthly test day record, the total regression equation could be used without compromising accuracy very much and with more computational ease. On the contrary, if the least desirable monthly test (the tenth) were used then the decision would probably be to use Procedure 2.

Since the cumulative test-day records are nearly as accurate as linear functions of the same test-day records for predicting total yield, the relative efficiencies of the sequential functions will be discussed only briefly, but are shown in Table 10. For the conventional situation when the last monthly records are lacking the regression procedure ignoring herd effects can apparently be used rather than the intra-herd procedure, because the difference in accuracy is relatively small. On the other hand, when the first months are missing the differences in accuracy of the two procedures are quite large unless four or five monthly tests are available.

The relative efficiencies of Procedures 1 and 2 when prediction is based on cumulative monthly test-day records are given in Table 11. These efficiencies follow the same pattern as those shown in Table 10. The efficiencies of the cumulative procedures when the last months are missing are similar, but when the first months are missing Procedure 2 is much more efficient. As was seen in Table 5, the average correlation between the yield predicted from a 5-mo. cumulative record and total yield is 0.93. The increase in average error of prediction over within-herd prediction is $(1176)^{\frac{1}{2}} - (1045)^{\frac{1}{2}} = 2.0$ lb. Therefore, it seems that prediction of total yield ignoring herd effects based on 5-mo. records is a possibility which could be considered for earlier proving of young sires.

Table 12 presents the relative efficiencies between Procedures 1 and 2 for linear functions of bimonthly and trimonthly test records. It is readily apparent that

TABLE 10

Relative efficiency of total and intra-herd regression for predicting total lactation yield from linear functions of sequential monthly test-day milk records [a]

	Sequential months of lactation—last months missing								
	1	2	3	4	5	6	7	8	9
V_1	4,585	2,638	1,914	1,378	930	654	441	190	51
V_2	3,774	2,293	1,664	1,226	839	598	410	182	50
E	121	115	115	112	111	109	108	104	102
	Sequential months of lactation—first months missing								
	10	9	8	7	6	5	4	3	2
V_1	6,446	4,784	2,965	1,789	1,159	727	346	166	65
V_2	4,082	3,164	2,130	1,384	949	622	306	149	60
E	158	151	139	129	122	117	113	112	108

[a] The linear functions corresponding to V_1 are given in Tables 3 and 4, and those corresponding to V_2 are shown in Tables 3 and 4 of VanVleck and Henderson (17).

TABLE 11

Relative efficiency of total and intra-herd regression for predicting total lactation yield from cumulative monthly test-day milk records [a]

	Cumulative months of lactation—last months missing								
	1	2	3	4	5	6	7	8	9
V_1	4,585	2,829	2,080	1,594	1,176	854	594	325	105
V_2	3,774	2,462	1,820	1,407	1,045	763	534	295	96
E	121	115	114	113	113	112	111	110	109
	Cumulative months of lactation—first months missing								
	10	9	8	7	6	5	4	3	2
V_1	6,446	5,292	3,995	2,794	1,871	1,191	645	281	86
V_2	4,082	3,396	2,644	1,927	1,354	906	512	232	75
E	158	156	151	145	138	131	126	121	115

[a] The regression coefficients corresponding to V_1 are given in Tables 5 and 6 and those corresponding to V_2 are shown in Tables 5 and 6 of VanVleck and Henderson (17).

TABLE 12

Relative efficiency of total and intra-herd regression for predicting total lactation yield from bimonthly and trimonthly test-day milk records [a]

	Sequential function					Cumulative				
	Bimonthly set		Trimonthly set			Bimonthly set		Trimonthly set		
	1	2	1	2	3	1	2	1	2	3
V_1	166	171	678	426	398	214	213	904	457	421
V_2	163	163	644	414	370	207	194	851	444	384
E	102	105	105	103	108	104	110	106	103	110

[a] The regression coefficients associated with V_1 are given in Tables 7 and 8 and those with V_2 are given in Tables 7 and 8 of VanVleck and Henderson (17).

there is little difference between the errors of prediction for the two procedures. Prediction of total yield ignoring herd effects from cumulative bimonthly tests appears to merit consideration as a replacement for testing every month. Testing costs for the herd owner could be approximately halved without any appreciable loss of accuracy in estimating total yield. This conclusion has previously been advanced by Gifford (7), McDowell (11), McKellip and Seath (12), and Alexander and Yapp (1). Bayley *et al.* (2) reported that bimonthly tests are about 96% as accurate as monthly tests, but cautioned "the frequency of the large errors indicates that bimonthly and quarterly records should be satisfactory for sire provings and population studies, but they may be unsatisfactory when used as individual lactation records."

CONCLUSIONS

The slight additional inaccuracy of predicting total milk yield from regression ignoring herd effects as compared with intra-herd regression does not appear as important as the extra computational difficulties encountered with intra-herd prediction for most practical situations. The practical situations considered include predicting total yield from five or more cumulative monthly test-day records, predicting total yield from cumulative bimonthly test-day records, and predicting total yield from a single test-day record obtained in the fourth, fifth, or sixth month of lactation.

The high correlations which exist between the total yield and cumulative monthly records 5 or more mo. in length and between total yield and cumulative bimonthly records strongly indicate the possibility of sire proving based on short records or on bimonthly records. The former could reduce the costs of proving young sires, increase genetic progress through a shorter generation interval or additional sampling, or provide a combination of these results, while the latter could reduce by about half the testing cost to herd owners.

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FERTILITY OF BULL SPERM WITH ADDED CATALASE[1]

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SUMMARY

Semen, from 44 bulls, that had been extended in glycerinated milk or Cornell University Extender (CUE), each with and without 1 γ/ml of added catalase, was used to inseminate 23,837 first-service cows, to measure the effects of catalase on fertility during storage for four days. CUE resulted in averages of 77.5, 74.8, 68.3, and 69.5% 60- to 90-day nonreturns for Days 1, 2, 3, and 4 of storage, respectively. The comparable averages for glycerinated milk were 73.9, 70.1, 65.9, and 56.2; for CUE with catalase were 78.2, 74.0, 69.8, and 68.1; and for glycerinated milk with catalase were 74.2, 74.9, 69.4, and 61.0.

Although the catalase had no appreciable effect on the fertility of sperm in CUE, this enzyme exerted the hypothesized beneficial effect upon the fertility of sperm in the milk extender. The failure to detect any improvement in fertility as a result of the addition of catalase to CUE is attributable to the inherent presence of detectable amounts of catalase activity in egg yolk. Any similar enzymatic activity inherent to milk is destroyed by the heat treatment of the milk.

The improved fertility observed as a result of addition of catalase to the milk extender is largely attributable to lowered embryonic mortality.

Contrary to the case for sea-urchin semen, which contains a relatively large amount of catalase (3, 7, 22), the negligible amount of catalase which has been reported to be present in bull semen (10, 24) is probably due to a contamination of the semen (15). The absence of catalase from bull semen is particularly important, because of the so-called oxygen damage to sperm which occurs as a result of oxygenation of semen (14, 29, 32). This oxygen damage is probably due to the accumulation of hydrogen peroxide from the oxidative deamination of the aromatic amino acids tyrosine, tryptophane, and/or phenylalanine (28).

VanDemark *et al.* found that added catalase apparently reduced the loss of motility of bull sperm which had been oxygenated (32), but observed no beneficial result with regard to fertility in an egg yolk–citrate extender (31). The majority of the matings in that experiment were performed with semen that was 24 to 36 hr. old. The author has found no report of the fertility of sperm in a milk extender with added catalase.

More recently, McLean (17), VanDemark *et al.* (33), Foote *et al.* (8, 9, 11), and others (1, 2, 18, 19, 26, 27) have described extenders for bull sperm that show considerable promise for maintenance of satisfactory fertility in bull sperm during storage at 5° C. for as long as 1 wk. Prolonged storage of sperm at 5° C. or higher temperatures may result in accumulations of hydrogen peroxide large enough to perceptibly affect fertility—accumulations larger than those that probably occurred in the fertility experiment by VanDemark *et al.* (31),

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in which sperm were stored for only one or two days. If this were true, inclusion of added catalase in the extender should result in improved fertility, with larger differences appearing as the storage interval increases.

The present investigation was designed to measure the fertility of bull sperm in a milk and in an egg yolk extender, each with and without added catalase, during four days of storage at 5° C.

EXPERIMENTAL PROCEDURES

Two routinely used semen extenders, a glycerinated milk extender and the Cornell University Extender (CUE), were selected to test the effects of added catalase [2] (1 γ/ml of extended semen), because of their widely different origin and composition. The glycerinated milk extender, described by McLean (17), was composed of homogenized whole milk that had been heated at 97 to 98° C. for 20 min., to which had been added 500 units of penicillin and 500 γ of streptomycin/ml. After cooling semen in this extender to 5° C., it was glycerinated, so that in final form it contained 10% (v/v) of glycerine. The CUE, described by Foote *et al.* (11), was composed of 20% egg yolk, 1.160% sodium citrate dihydrate, 0.168% sodium bicarbonate, 0.032% potassium chloride, 0.240% glucose, 0.240% sulfanilamide, 0.750% glycine, 0.087% citric acid, and 500 units of penicillin and 500 γ of streptomycin per milliliter.

To provide a broader base from which to predict future results, the experiment was performed for four consecutive weeks in a relatively cold month, March, with a mean daily temperature of 22.8° F. (30), then repeated in a relatively warm month, August, with a mean daily temperature of 68.8° F. (30). The number of first services that were expected in these two periods, and the error variance estimated by Dunn *et al.* (6), were used to estimate that this number of services would be sufficient to detect a difference at least as small as two percentage units in 60- to 90-day nonreturn rates at the 5% level of significance, with a 95% chance of detecting the difference in a two-treatment experiment.

The bulls included in the experiment were those routinely used at Michigan Artificial Breeders Cooperative. Each bull was ejaculated once weekly, except when a second ejaculate was needed to provide a number of sperm sufficient to meet the requirements of the Cooperative, in which case the first and second ejaculates were composited. The extension medium used (milk or CUE, either with or without catalase) for each bull during each 4-wk. period of the experiment was determined at random, with the condition that each of the four media was used once during each 4-wk. period.

To approach an equal distribution of services among the four days of storage, and to meet the operational requirements of the Cooperative, the extended semen was shipped to the breeding technicians twice weekly. This shipment schedule was expected to result in about half the number of services on the fourth day of storage as were expected on each of the first three days of storage.

[2] Twice-crystallized beef liver catalase, Nutritional Biochemicals Corp., Cleveland, Ohio.

Cows were inseminated with about 1 ml. of extended semen containing at least 20 million sperm per milliliter.

The operational routine of the Cooperative determined that the extended semen was first used for inseminations about 24 hr. after collection. Consequently, in the present inquiry, the first, second, third, and fourth days of storage of the semen represented sperm stored for about 24 to 36, 48 to 60, 72 to 84, and 96 to 108 hr., respectively.

The analyses of variance of the data considered the following sources of variation: extender, catalase, day of storage, period, bull, and 18 appropriate interactions among these five main effects. The extender, catalase, day of storage, and period sources of variation were considered to be fixed and factorial; whereas, the bull source was considered to be random and nested within period.

So as to avoid the excessive variability experienced with fertility estimates based on very few services, only those bulls with at least five services on each day of storage in each extension medium were used in the statistical analyses of the data. Identical analyses were made on the 60- to 90-day nonreturn percentages and on the declines from 30- to 60- to 60- to 90-day nonreturn percentages, the latter criterion being taken as a measure of embryonic mortality (23).

RESULTS

A total of 44 bulls, 29 Holsteins, seven Guernseys, one Jersey, three Herefords, and four Angus, with a total of 23,837 first services, qualified for the statistical analyses of the data. The March period included 24 bulls with 14,855 services, and the remaining 20 bulls with 8,982 services were included in the August period. The distribution of first services among the four extension media is listed in Table 1 by day of storage.

TABLE 1

Number of first services with sperm in each extension medium

Day of storage	No added catalase		1 γ/ml added catalase		Total
	CUE	Milk	CUE	Milk	
1	1,602	1,837	1,749	1,720	6,908
2	1,739	1,818	1,703	1,657	6,917
3	1,769	1,421	1,795	1,446	6,431
4	954	868	996	763	3,581
Total	6,064	5,944	6,243	5,586	23,837

The average 60- to 90-day nonreturn percentages are shown in Table 2 for sperm in each extender, without and with added catalase by day of storage. The average of 72% 60- to 90-day nonreturns for sperm in the milk extender on Days 1 and 2 of storage is in very good agreement with recent experience with this extender under routine stud conditions at the Michigan Artificial Breeders Cooperative. Furthermore, the daily 60- to 90-day nonreturn averages shown for sperm in CUE (Table 2) are in general agreement with comparable data obtained in New York (8).

TABLE 2

Average 60- to 90-day nonreturn percentages for sperm in each extension medium

Day of storage	No added catalase		1 γ/ml added catalase	
	CUE	Milk	CUE	Milk
1	77.5	73.9	78.2	74.2
2	74.8	70.1	74.0	74.9
3	68.3	65.9	69.8	69.4
4	69.5	56.2	68.1	61.0
Av.	72.9	68.0	73.2	71.2

Analysis of variance of the 60- to 90-day nonreturn percentages, summarized in Table 3, revealed that the average of 73.0% 60- to 90-day nonreturns for sperm in CUE was significantly higher than the 69.6% for sperm in milk ($P < .001$). Addition of catalase to the extenders resulted in an average of 72.2% nonreturns, significantly higher than the 70.5% for the extenders without added catalase ($P < .05$). As expected, fertility declined considerably during storage ($P < .001$), but the two periods (months) did not differ appreciably ($P \cong .24$). The interaction between extenders and days of storage was significant ($P < .001$), illustrative of the fact that the fertility of sperm in the milk extender declined 15.5 percentage units (from 74.1 to 58.6%) during the four days of storage; whereas, that of sperm in CUE declined only 9.1 percentage units (from 77.9 to 68.8%). The interaction between extenders and levels of catalase was significant ($P \cong .17$), pointing to the fact that catalase had

TABLE 3

Summary of analysis of variance of 60- to 90-day nonreturn percentages

Source of variation	Degrees of freedom	Mean square	F ratio	Probability (P_1)
Extender (E)	1	1,869	12.71	< 0.001
Storage day (S)	3	3,749	378.69	< 0.001
Catalase (C)	1	450	4.95	< 0.05
Period (P)	1	648	1.42	$\cong 0.24$
Interaction (ES)	3	430	6.32	< 0.001
(EC)	1	328	2.12	$\cong 0.17$
(EP)	1	435	2.96	$\cong 0.09$
(SC)	3	31	0.32	> 0.50
(SP)	3	91	0.92	$\cong 0.45$
(CP)	1	3	0.03	> 0.50
(ESC)	3	89	1.25	$\cong 0.31$
(ESP)	3	67	0.99	$\cong 0.42$
(ECP)	1	456	2.94	$\cong 0.10$
(SCP)	3	47	0.48	> 0.50
(ESCP)	3	103	1.45	$\cong 0.24$
Bulls (B: P)	42	455	No appropriate F denominator	
Interaction (EB: P)	42	147	No appropriate F denominator	
(SB: P)	104	99	No appropriate F denominator	
(CB: P)	42	91	No appropriate F denominator	
(ESB: P)	104	68	No appropriate F denominator	
(ECB: P)	42	155	No appropriate F denominator	
(SCB: P)	104	98	No appropriate F denominator	
(ESCB: P)	104	71	No appropriate F denominator	

almost no measurable effect upon the fertility of sperm in CUE; whereas, the addition of catalase to the milk extender resulted in an average improvement of 3.2 percentage units (68.0 vs. 71.2%) in the nonreturn rate of the sperm.

Unexpectedly, the observed fertility of the sperm in the milk extender for the March period was 67.9%, significantly lower than the 71.6% for August ($P \cong .09$), but there was no significant difference between the 72.9% for CUE in March as compared to the 73.3% for CUE in August. The extender-catalase-period interaction was significant ($P \cong .10$), indicating that while the addition of catalase to milk resulted in an improvement in fertility in both periods, addition of catalase to CUE during March resulted in a slight (1.4%) decrease in fertility and a similar addition during August resulted in a slight increase (2.3%) in fertility. None of the other interactions among the treatment effects approached significance.

TABLE 4

Average declines from 30- to 60- to 60- to 90-day nonreturn percentages for sperm in each extension medium

Day of storage	No added catalase		1 γ/ml added catalase	
	CUE	Milk	CUE	Milk
1	6.0	6.9	6.5	6.4
2	6.3	8.3	5.4	5.7
3	7.3	7.9	6.6	5.7
4	7.2	10.1	8.2	7.5
Av.	6.6	8.0	6.5	6.2

Average values for embryonic mortality, as measured by decline in fertility from 30- to 60- to 60- to 90-day nonreturn rates, are tabulated in Table 4 for each extender without and with added catalase by day of storage. These data show that the addition of catalase had no consistent effect upon embryonic mortality resultant from matings with sperm in CUE, but there resulted an apparent drop in embryonic mortality when catalase was added to the milk extender ($P \cong .25$), an improvement which appeared to be magnified as storage time increased. Analysis of variance of these data showed no significant difference between the two extenders ($P > .40$). The four days of storage, however, differed significantly ($P < .01$).

Addition of catalase resulted in an average decline of 6.3%, appreciably less than the 7.3% for no catalase ($P \cong .11$). The beneficial effect of catalase upon this measure of embryonic mortality was confined to the March period ($P \cong .08$), when semen without catalase averaged 8.1% decline and semen with added catalase averaged 6.1%, as compared to 6.4 and 6.5%, respectively, for the August period. The full meaning of this result is not clear. None of the other interactions among the treatment effects approached significance.

DISCUSSION

A comparison of Tables 2 and 4 indicates that about half of the improvement in fertility observed when catalase was added to the milk extender was a result

of lowered embryonic mortality. This observation indicates that the accumulation of hydrogen peroxide during storage of bull spermatozoa contributes to sperm senescence (16).

The failure to detect any improvement in fertility when catalase was added to CUE is in agreement with the results of VanDemark *et al.* (31), who tested fertility in an egg yolk extender. On the other hand, the observed improvement in fertility when catalase was added to the milk extender fits the hypothesis stated in the introduction above. Both the egg yolk in CUE, and milk, contain abundant amounts of each of the three aromatic amino acids (12, 21) that may result in formation of hydrogen peroxide by oxidative deamination.

Two explanations were advanced to reconcile these differing results. Either there is an inhibitor of catalase in egg yolk extenders, or catalase is inherently present in egg yolk. The first alternative explanation seemed to be substantiated by the claim of Shinn *et al.* (25), that sulfanilamide may exert its observed effects through inhibition of catalase, but Desjardine and Hafs (5) have recently rejected this idea on the basis of their ability to detect small amounts of added catalase (1 γ/ml of extender) throughout 1 wk. of storage in CUE. The second explanation seemed to be substantiated by Kaufman (13), who was able to detect weak catalase activity in egg yolk by a eudiometric assay. Although eudiometric assays are quite inaccurate and highly nonspecific (4), the presence of catalase activity in fresh hens' egg yolk has recently been verified (5) by a much more specific assay. Thus, when a small amount of catalase was added to an egg yolk extender, no perceptible improvement in fertility should have been expected.

This explanation for the conflicting fertility results obtained when catalase was included in CUE, as compared to those obtained when catalase was added to the milk extender, could be true only if there was no inherent catalase or peroxidase activity in the milk used for extending semen. It is well known that raw milk contains a small amount of catalase which is associated with the leucocytes contained therein and, in addition, a very large amount of lactoperoxidase (12). Since milk used for semen extension is first clarified in the processing plant, the leucocytes have been removed. Desjardins and Hafs have concluded that the considerable amount of catalase remaining in milk after clarifying is totally inactivated by heating at 94° C. for 10 min. (unpublished data). They found no regeneration of activity after this heat treatment. According to Pien (20), the lactoperoxidase in milk is totally inactivated by heating at 80° C. for 3 min., a heat treatment considerably less severe than that used in the treatment of milk preparatory to its use as a semen extender. Thus, when catalase was added to a milk extender, no other similar enzymatic activity would have masked its effect.

We may conclude that the addition of catalase to the milk extender resulted in decided fertility improvement, especially as the storage interval increased, and that the failure to detect such improvement with sperm in an egg yolk extender was a result of the inherent presence of catalase in egg yolk.

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REPRODUCTIVE CAPACITY OF DAIRY BULLS. I. TECHNIQUE FOR DIRECT MEASUREMENT OF GONADAL AND EXTRA-GONADAL SPERM RESERVES [1]

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SUMMARY

A technique for determining the gonadal and extra-gonadal sperm reserves of bulls by direct counting is described. Sperm were liberated from the epididymis by thoroughly tearing apart the tissue in 50 ml. of 0.9% NaCl and grinding the tissue suspension in 200 to 500 ml. of saline for a total of 2.5 min. in a Waring Blendor. After removing the tunica albuginea, testicular sperm were liberated by cutting the testis into small pieces and grinding in 1,000 ml. of saline for 2 min. Sperm were liberated from the ductus deferens and ampulla by flushing with distilled water to remove most of the sperm and then grinding in a blendor. Soaking the sperm suspensions for 48 hr. before counting did not result in higher values; differences between 0- and 48-hr. counts were relatively small. Sampling of the sperm suspension is the major source of error; therefore, replicate counts should be made on separate subsamples of the entire suspension.

The extra-gonadal sperm reserves (EGR) of bulls have been estimated indirectly by depletion trials (2, 3, 19). In the absence of direct determinations of EGR, however, the relationship between the total number of sperm collected in a depletion trial and actual EGR could not be established (3). Knowledge of the extent of EGR also is necessary to determine how many sperm must be collected before any demand is placed on testicular production of sperm when daily sperm output is used to estimate daily sperm production (9).

Several workers have utilized flushing techniques for removing epididymal sperm. Except for the work reported by Bialy and Smith (4), these attempts were qualitative not quantitative. When the present studies were begun in January 1956, only brief descriptions of the techniques for determining the EGR of boars (13), rams (5, 14, 17), and rabbits (8, 12) were available. Thus, the technique developed at this Station was based partly on methods used for the extraction of somatic cell nuclei in enzyme studies. Stoneburg (18) found that cell nuclei could be liberated from muscle tissue by treatment with citric acid, filtration, centrifugation, and enzymatic digestion. Dounce (6) elaborated on Stoneburg's technique and suggested pulverizing frozen liver or tumor tissue in a Waring Blendor for 10 to 15 min. in a suspension medium of M/475 or M/14 citric acid. He later reported that 0.9% NaCl gave equally as good results (7). Ortavant (16) used this method as basis for determining the EGR of rams. Hoerr (10) criticized Dounce's technique on the basis that the Waring Blendor destroyed many somatic cell nuclei during the 10-min. treatment period.

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Experience in this laboratory with bull spermatozoa indicates that it is virtually impossible to destroy the sperm head, especially by relatively short grinding periods in a Waring Blendor, because of the condensed nuclear chromatin.

Using maceration, grinding, centrifugation, and filtration, Ortavant (16) obtained an almost pure suspension of epididymal sperm. This procedure is quite involved and sperm losses are apt to occur during centrifugal separation of the larger tissue debris and subsequent filtration. Although Bialy and Smith (4) reported that a flushing technique was superior to their maceration and grinding technique, the values reported appear to be very low.

Because of the importance of accurate EGR determinations in bulls (3, 9, 14), and the wide discrepancies among values in the literature (2, 4, 15), the technique developed in our laboratory is described and compared with other techniques.

GENERAL PROCEDURE

Dairy bulls 2 to 12 yr. of age with a known reproductive history were trucked to a local abattoir (30 mi.). Upon arrival, the bulls were stunned with a rifle shot, the carotid arteries severed, and the head removed. In a few bulls muscular contractions caused emission of seminal fluid. These contractions also may have altered the distribution of sperm within the reproductive system. The reproductive system was carefully removed in toto by opening the abdomen, severing the ischial arch, and loosening the supporting tissue. Upon return to the laboratory, the various components of the system were freed of extraneous tissue, weighed, and measured. Testis volume, including the tunica albuginea but not the epididymis, was determined by displacement in 0.9% NaCl.

Approximately 4 hr. after death, the reproductive system had been sectioned into its component parts. The exact points of division are of prime importance, since they affect the relative sperm distribution. The majority of these cuts were made by one person and the exact locations were based on gross anatomical structure and tubular size. The individual portions of the reproductive system, except the testes, were placed in 6-in. evaporating dishes and covered with moist paper towels to retard tissue dehydration.

Using a maceration and grinding technique, the sperm contained in each portion of the reproductive system were liberated into sterile, aqueous 0.9% NaCl at room temperature. Particular care was used to avoid the loss of sperm and the saline utilized to rinse all equipment was added to the respective sample. Preliminary investigation demonstrated that sperm counts were not affected either by (a) grinding ejaculated sperm diluted in distilled water (2 ml. of semen and 400 ml. of water) for 2 min. in a Waring Blendor or (b) grinding a similar sample mixed with washed connective tissue for 2 min.

All suspensions were treated with a mixture of penicillin and dihydrostreptomycin before storage to retard bacterial growth.

LIBERATION OF EPIDIDYMAL SPERM

The caput, corpus, and cauda epididymis were placed in separate evaporating dishes and thoroughly torn apart with a scalpel and forceps in 50 ml. of

saline, to break down the connective tissue of the saccus epididymis and tubule wall. After soaking for 1 to 2 hr., the macerated tissue was placed in the 1-qt. jar of a Waring Blendor. More saline was added for the initial blending, the volume varying with the portion of the epididymis and the preslaughter treatment (1) of the bull. The approximate volumes are shown in Table 1 and are

TABLE 1

Approximate volumes of 0.9% NaCl used for liberating sperm from portions of the reproductive system

Portion of reproductive system	Vol. of saline for each blending	Final vol. for counting
	(ml.)	*(ml.)*
Testis	500	3,000
Caput epididymis	400	1,000–2,500
Corpus epididymis	200	500–600
Cauda epididymis	500	1,200–5,000
Ductus deferens	50[a]	100
Ampulla	50[a]	100

[a] The supernatant fraction for the ductus deferens and ampulla was prepared by flushing with 100 ml. of distilled water and only the second fraction prepared by grinding for 2 min.

considerably larger than those used by other workers (4, 11, 16). After grinding for 30 sec. at approximately 10,000 r.p.m., all but 100 ml. of the supernatant was removed with a 50-ml. volumetric pipette and placed in an Erlenmeyer flask. Pieces of tissue remaining in the blendor jar were grasped with forceps and cut into very small pieces. Saline was added to restore the original volume and the mixture ground for 2 min.

Initially, the second suspension was counted separately from the supernatant. The first and second suspensions of the caput and cauda of four epididymides were counted separately and after being combined. Adding the separate counts yielded an average of 9.30 for the caput and 13.14 billion sperm for the cauda, as compared to 9.33 and 13.40 billion when the combined fractions were counted. Thus, the accuracy of the counts on the supernatant fraction which contained the majority of the sperm was not lessened by the addition of the second fraction containing the connective tissue debris. Thereafter, the two fractions were combined for counting to save time. In some instances, additional saline was added to reduce sperm concentration to a level suitable for counting.

During storage for 48 hr. at 5° C., the epididymal suspensions were mixed every 6 to 8 hr. The counting procedure is described below. The differences in the average counts initially and after 48 hr. of soaking are shown in Table 2. Although the differences for the testis and caput epididymis were significant ($p < 0.05$), they were rather small. This is in marked contrast to the report of Ortavant (16) on ram epididymides. Using a different preparative technique, he found that maximum numbers of sperm were counted in suspensions of the caput or cauda epididymis after 96 and 72 hr. of soaking, respectively. How-

TABLE 2

Effect of soaking at 5° C. for 48 hr. after grinding on the number of sperm in various portions of the reproductive system [a]

(Billions of sperm)

Portion of reproductive system	No. of samples	Av. No. of sperm after		Diff.
		0 hr.	48 hr.	
Testis	15	17.50	16.08	−1.42 [b]
Epididymis				
Caput	22	6.00	5.75	−0.25 [b]
Corpus	9	1.53	1.49	−0.04
Cauda	21	9.66	9.76	+0.11
Average	52	6.70	6.63	−0.07

[a] The sperm counts do not represent actual values for that portion of the reproductive system as some of the 0- and 48-hr. comparisons involved only one of the two fractions initially prepared.

[b] Significant at the 5% level.

ever, he did not make control counts before soaking. Ortavant concluded that a grinding time of 1 min. at 12,000 r.p.m. followed by 48 to 96 hr. of soaking at 5° C. gave best results. Working with boars, Kennelly (11) found no difference between 48- and 96-hr. counts where the same method of sample preparation was used. At these time intervals, the epididymal suspensions were mixed for 15 sec. in a Waring Blendor before removing the sample for counting. The sample for the 0-hr. counts, however, was removed after the suspension had settled for 15 min. This difference in technique may explain why he obtained significantly lower values at 0 hr. than at 48 and 96 hr. Based on the small differences between 0- and 48-hr. counts (Table 2), for convenience all subsequent epididymal and testicular suspensions were counted after 48 hr. of storage at 5° C.

LIBERATION OF SPERM FROM THE DUCTUS DEFERENS AND AMPULLA

Sperm in the ductus deferens were flushed out with 100 ml. of distilled water, using a syringe and hypodermic needle held in place with a hemostat. This technique was practical because of the relatively large diameter of this duct. The suspension was collected in a flask and counted after proper dilution. The flushed ductus deferens was cut into pieces about 1-cm. long and placed in the blendor with 50 ml. of saline. After 30 sec. of grinding, tissue remnants were cut into small pieces and the mixture ground for an additional 2 min. The suspension was poured into a second flask and counted separately; it contained 1 to 23% of the total sperm in the ductus deferens.

Spermatozoa present in the ampulla were liberated in a similar manner, except that prior to grinding the ampulla was split longitudinally, macerated in 50 ml. of saline, and ground in 50 ml. of saline.

LIBERATION OF SPERM FROM THE TESTIS

The testis was cut in half longitudinally and the tunica albuginea carefully removed, starting at the dorsal pole. Contrary to the difficulty reported with

boar testes (11), essentially no testicular tissue adhered to the tunic. After cutting into 1- to 2-cm. cubes, half of the testis was placed into the blendor and 500 ml. of saline added. The mixture was ground for 1 min., allowed to settle for 1 min., and ground for another minute. The essentially homogeneous suspension was placed into a 12-qt. polyethylene pail and the blendor jar rinsed with 1,000 ml. of saline which was added to the suspension. The other half of the testis was treated in the same manner, so that the entire testis was suspended in a total of 3,000 ml. of saline. This volume was found to be satisfactory for all but very large testes. The pail was covered and stored at 5° C. for 48 hr. and the suspension mixed at 6- to 8-hr. intervals.

COUNTING PROCEDURE

The sperm present in the suspensions of ductus deferens and ampulla were counted on the day of slaughter, whereas those of the testis and epididymis were counted after 48 hr. of storage at 5° C. Using phase contrast microscopes, the suspensions were counted in A. O. Spencer Bright-Line haemacytometers at dilutions which resulted in about 80 to 120 spermatozoa per 80 small squares.

In most cases, each suspension was counted by four observers. After thoroughly mixing the suspension in the flask, each observer filled one chamber of the slide, using a 0.1-ml. serological pipette. Following remixing, a second subsample was used to fill the other chamber. The four diagonals, each composed of 80 small squares, in the two chambers were counted and if the four values did not agree within 10% of the highest value the observer prepared a second slide. Individual values for 80 small squares were averaged (average of 16 to 20 counts) and, using the appropriate dilution factor, the total sperm for each portion of the tract was determined. The small volume contributed by the tissue to the total volume was ignored except for the testis. By adding testis volume (minus 8.7% for the volume of the tunica albuginea) to the volume of saline used, testis sperm counts were corrected for the volumetric contribution by the testis.

Counts for the cauda epididymis were used to estimate the components of variance in the counting procedure. Of the more than 100 cauda counted, only 17 were counted by the same four technicians. For this analysis, the first series of four counts by each of the four technicians was used, even though 9.6% of

TABLE 3

Sources of variation in counting procedure

Source	d.f.	M.S.	Components of variance	Per cent of variance
Epididymides (cauda)	16	5,980[a]	364	81
Technicians	3	150	0	0
Epididymides × technicians	48	159[a]	22	5
Samples within technicians and epididymides	68	71	8	2
Counts within samples	136	55	55	12

[a] Significant at the 1% level.

the 272 counts in Table 3 eventually were excluded because they exceeded the limit of 10% variability described above. The importance of this culling procedure is emphasized by the highly significant epididymides × technicians interaction. With the technique used for mixing and sampling the sperm suspension, sampling error was only 2% of the total variance (9% of the within epididymides variance). The need for counting both diagonals of a chamber is suggested by the 12% variance found for counts within samples (chambers).

At the time this work was started, it was decided that each chamber of the haemacytometer should be prepared with a different subsample from the entire sperm suspension. This procedure appears to be greatly superior to that of Ortavant (16) and Kennelly (11), in which a 1-ml. sample of the sperm suspension was diluted either 1:9 or 1:19 and all counts made on this single sample. Regardless of the number of replicate counts made from such a sample, the accuracy can be no greater than that of the single sampling of the entire suspension. These authors also appear to have made fewer replicate determinations of a given sperm suspension.

The recognition of sperm cells presented a problem only for testicular suspensions. For the latter, only spermatozoa and elongated (Stages VI to VIII) spermatids were counted. Some practice was required to differentiate these cells from the tissue debris present.

With the technique described in this paper, the EGR of 35 mature bulls averaged 72.6 billion sperm and the gonadal sperm reserve averaged 54.8 million sperm per gram of testicular tissue. The detailed results will be reported later (1).

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KINETICS OF SUBCUTANEOUS ABSORPTION OF L-THYROXINE-I^{131} AS MEASURED BY INCREASE OF I^{131} IN BLOOD OF CATTLE [1]

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SUMMARY

Three hundred microcuries of I^{131}-labeled thyroxine were injected subcutaneously into Holstein and Jersey cattle. At frequent intervals thereafter, I^{131} activity was measured both in the blood and in the thyroid gland. Half an hour after injection, I^{131} could be detected in blood. The over-all 24-hr. absorption was exponential in character and could be described by a faster and a slower component. The range in half-time ($t\frac{1}{2}$) for appearance of radioactivity in blood was 1.5-2.2 hr., with a mean of 1.82 and 1.92 hr., respectively, for Holstein and Jersey cattle. In most of the animals the period of rapid increase in blood-I^{131} was complete within 6-10 hr. after thyroxine-I^{131} administration. Thyroidal-I^{131} determination indicated that I^{131} from the metabolism of thyroxine began to reach the thyroid gland within a period of 4 hr. The investigation revealed a gradual and yet sufficiently rapid increase in blood-I^{131} to simulate the normal physiological rate of thyroxine secretion. It is concluded that estimation of daily thyroxine secretion rate as determined by a replacement therapy method would not be subject to error by the rate of absorption of thyroxine from the skin.

Several investigators (2-4) have estimated the daily thyroxine secretion rate in cattle by substitution techniques that have involved the replacement of endogenous thyroxine by an equal amount of exogenous thyroxine. This replacement has invariably been made by the subcutaneous route. If the absorption of thyroxine by this path was either very slow or rapid, then the possibility would exist for an over- or under-estimation of thyroxine secretion rate. It was of value, therefore, to determine the time course of absorption of L-thyroxine in cattle when injected subcutaneously as measured by increase of I^{131} in blood. The present paper describes such observations.

EXPERIMENTAL PROCEDURE

Mature Jersey and Holstein cattle were kept in a dry lot and had free access to iodized salt; 300 μc. chromatographically pure I^{131}-labeled thyroxine in a volume of 0.6-0.8 ml. in a 50% propylene glycol carrier were injected subcutaneously in each animal, either in the shoulder or in the hip region. At frequent intervals, blood samples were drawn from the jugular vein and radioactivity measured in a National Radiac Scintillation well counter (Model 5A-2D). In some experiments 4 g. tapazole/1,000 lb. body weight were administered in

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gelatin capsules one day prior to subcutaneous injection, to prevent the reutilization of I^{131} of metabolized thyroxine-I^{131} (7). The thyroidal-I^{131} in vivo counts were made by methods described by Pipes *et al.* (5). After subcutaneous thyroxine-I^{131} injection, the half-time for increase in radioactivity ($t\frac{1}{2}$) in blood was calculated by the equation $\frac{1n2}{m}$ (1), where $1n$ is the natural logarithm and m is the slope of the regression line (i.e., per cent dose against time).

RESULTS

Appearance of thyroxine-I^{131} in the blood after subcutaneous absorption. In all normal animals the radioactivity could be detected in blood one-half hour after subcutaneous injection of I^{131}-labeled thyroxine. There was a steady increase in radioactivity with time, and when the log of the activity was plotted against time a straight line could be described, indicating an exponential nature of increase in blood-I^{131} (Figure 1). The half-time for increase in blood radioactivity ($t\frac{1}{2}$) varied between 1.5-2.2 hr. in different animals. The average for the group of Holstein cattle was 1.82 hr., and of Jersey cattle was 1.92 hr. However, after 6 hr. the maximum rate of increase of blood-I^{131} had occurred and a second, slower component of increase began, with a $t\frac{1}{2}$ of 12.4 and 11.9 hr. for Holstein and Jersey cattle, respectively. The over-all absorption of L-thyroxine as measured by increase of blood-I^{131} could, therefore, be described by two equations:

$$\log y = 10^{-2}\,(6 \times -2) \quad \text{(fast component)}$$
$$\log y = 10^{-2}\,(3 \times +22) \quad \text{(slow component)}.$$

The rapid phase was practically complete by 8-10 hr. in different animals and the increase in radioactivity in successive measurements after 10 hr. was smaller, reaching equilibrium within 24 hr. The difference in $t\frac{1}{2}$ between breeds was not more than within the same breed.

The half-time for increase in blood radioactivity after subcutaneous injection of I^{131}-labeled thyroxine in tapazole-treated animals was 25% longer than in nontreated animals (Table 1 and Figure 1). The regression equations for slower and faster components of increase of I^{131}-labeled thyroxine in the blood were

$$\log y = 10^{-2}\,(5 \times +4) \quad \text{(fast component)}$$
$$\log y = 10^{-2}\,(2 \times +25) \quad \text{(slow component)}.$$

TABLE 1

Half-time ($t\frac{1}{2}$) for increase in appearance of I^{131} in blood after subcutaneous injection of I^{131}-labeled thyroxine

No. of animals	Breed	Faster component of absorption	Slower component of absorption
		With tapazole	
4	Holstein	2.46 ± 0.72	14.37 ± 1.30
3	Jersey	2.36 ± 0.53	13.90 ± 1.12
		Without tapazole	
5	Holstein	1.82 ± 0.3	12.4 ± 1.60
4	Jersey	1.92 ± 0.5	11.9 ± 1.31

THYROIDAL-I^{131} RADIOACTIVITY

External thyroidal-I^{131} counts made at hourly intervals concurrently with I^{131} determinations in blood revealed a gradual increase, beginning 4 hr. after subcutaneous thyroxine-I^{131} injection. A definite increase (at least by 25% above background) in thyroidal-I^{131} was observed 8 hr. after thyroxine-I^{131} administration and, thereafter, the half-time for increase in radioactivity was 12 ± 1.5 hr. (eight animals). This would, therefore, indicate that approximately 4 hr. are required for part of the subcutaneous injected thyroxine to be metabolized in the cells (either at site of injection or somatic) and for the I^{131} liberated in the cells to enter the blood stream and to begin to be picked up by the thyroid gland.

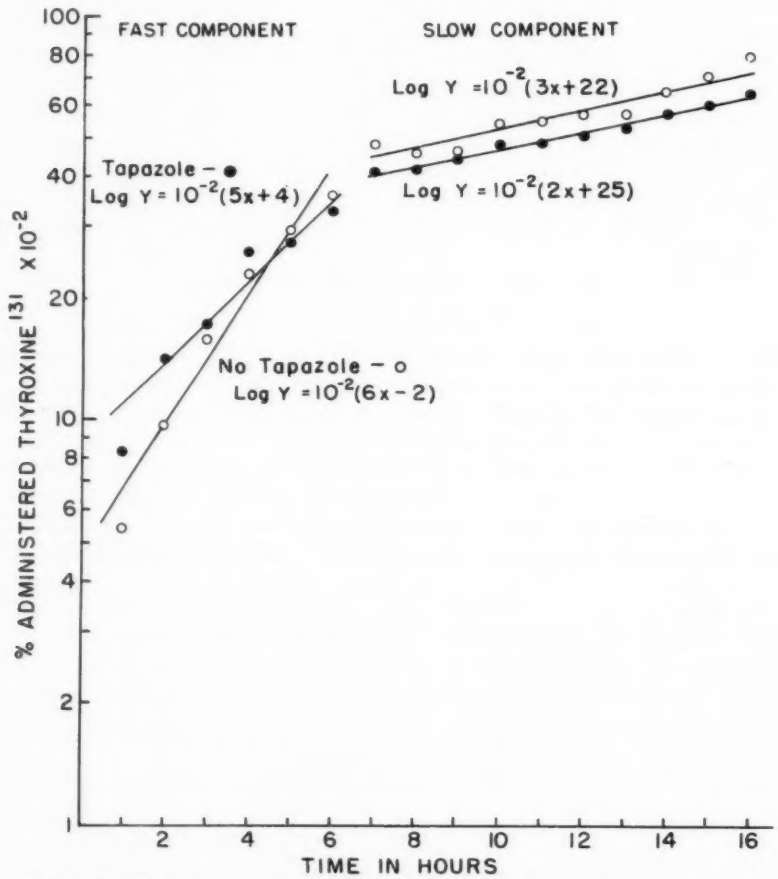

FIG. 1. Kinetics of subcutaneous absorption of L-thyroxine as measured by increase of I^{131} in blood of normal and tapazole-fed cattle. Both groups showed an early fast exponential increase for about 8 hr., followed by a slower increase, reaching equilibrium within 24 hr.

DISCUSSION

The time course of subcutaneous absorption of thyroxine as measured by increase of I^{131} in blood revealed in the present study represents a gradual phenomenon involving a faster and a slower component. By 24 hr. the absorption is believed to be essentially complete as indicated by the plateau of I^{131} in the blood. If additional thyroxine-I^{131} is being absorbed from the injection site after this time, it is occurring at a rate equal to or less than the rate of elimination of I^{131} from injected thyroxine I^{131} ($t\frac{1}{2} = 2.47$ days). The importance of this observation can not be over-emphasized in its relation to daily thyroxine secretion rate estimation. If the subcutaneous injection of thyroxine was followed by its rapid entry into the blood stream, this would represent an unphysiological phenomenon in relation to normal thyroid gland secretion. Since the disappearance of thyroxine-I^{131} from the blood is faster in animals maintained on an elevated thyroxine level (6), a sudden total absorption might result in a faster disappearance of the hormone and, therefore, the estimated secretion rates obtained would probably not be representative of true thyroid secretion. On the other hand, a very slow rate of absorption from the skin would mean smaller amounts of exogenous hormone in the blood stream and, consequently, the inhibition of thyrotropin (TSH) output would also be less. Therefore, larger amounts of hormone might be required to bring the thyroxine level of the blood up to the point to prevent TSH output. Therefore, a relatively rapid and yet gradual absorption of the hormone into the blood stream as revealed by the present study would compare favorably with the rate of secretion by the thyroid gland.

In this study, the initial increase in radioactivity in the blood represents the absorption of thyroxine-I^{131} from the skin. However, as thyroxine-I^{131} is metabolized (deiodinated) in the tissue, free I^{131} is returned to the blood. In the normal animal, the free I^{131} is removed very rapidly from the blood, both by excretion through the kidney into the urine and by its uptake by the thyroid gland. It is believed that free I^{131} contributes only slightly to the total radioactivity measured in the blood, due to the rapidity of its removal by the kidney and uptake by the thyroid gland. On the other hand, thyroxine-I^{131} absorbed into the blood from the injection site is metabolized very slowly as indicated by the mean $t\frac{1}{2}$ of 2.47 days in cattle. In animals administered a goitrogen, the part normally picked up by the thyroid gland would be excreted in the urine also.

In the present experiment, it was shown that detectible amounts of I^{131} were being picked up by the thyroid glands of the normal animals within 4 hr. after injection of thyroxine-I^{131}. This indicates that about 4 hr. are required for thyroxine injected subcutaneously to reach the cells and for free I^{131} to begin to be returned to the blood. During this period (4 hr.) there should be little or no difference between the two groups of cows in the rate of appearance of I^{131} in blood from the site of injection, since thyroid I^{131} uptake block by goitrogen would not begin to function until free iodine from the metabolism of thyroxine should begin to appear, yet the difference between the two groups is most marked during this period. While prevention of recycling metabolized

I^{131} by the goitrogen (tapazole) might explain differences in absorption rate between normal and treated animals 4-8 hr. after subcutaneous thyroxine-I^{131} administration, the precise role of tapazole in early (within 4 hr.) absorption differences noted is not clear. It is possible that tapazole in some way facilitates the early absorption of thyroxine-I^{131} from the subcutaneous tissue and thus accounts for the higher blood levels during the first 4 hr. and consistently slightly lower levels subsequently.

The differences in individual rate of flow of thyroxine from the subcutaneous tissue into the blood may be due to (1) the vascularity of the tissue, (2) the extent of fat deposition, (3) the presence of lymph capillaries, and (4) factors influencing adrenaline secretion which might constrict the capillary blood.

The over-all 24-hr. subcutaneous absorption of thyroxine as measured by increase of I^{131} in blood is sufficiently rapid so that little carry-over of thyroxine exists between successive injections; nor is it too rapid to disturb the metabolism of thyroxine. Assuming that the normal secretion of thyroxine is at a fairly uniform rate, the subcutaneous absorption of thyroxine would simulate the physiological phenomenon. It may be assumed on the basis of these observations that estimation of daily thyroxine secretion rates by replacement techniques are not in appreciable error because of factors involved in subcutaneous absorption.

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ERRATUM

Effect of Complex Mineral-Vitamin Supplement on Milk Production. Page 501, March Journal, 1961. Table 3, first column, should be "ENE from roughage %."

TECHNICAL NOTES

A STAINLESS STEEL ESOPHAGEAL-FISTULA CANNULA FOR DAIRY CATTLE NUTRITION STUDIES[1]

In nutrition studies, several methods for collecting forage samples for chemical determinations have been used. Clipping of forages has been most extensively used. This method does not give an accurate picture of the forage actually consumed by the animals, especially those grazing on mixed and range pastures, because of animal preference for some plants over others and their habit of grazing the plants at random heights. Collection of forage samples by closely observing what the animal grazes, and hand-plucking a comparable sample, has also been used. These methods are time-consuming and tedious and the forage samples obtained only approximate the feed actually eaten.

The use of the esophageal-fistula first proposed by Torell (2) for grazing sheep offered a new approach for collecting forage samples. The esophageal fistula was closed and opened by the use of stainless steel pins. Cook *et al.* (1) successfully adapted a plastic cannula of 1-in. diameter, permanently installed in the esophageal-fistula of sheep. Since 1959 we have conducted studies on the insertion and permanency of several types and sizes, 1-1½ in.-diameter, plastic esophageal-fistula cannulae for dairy cows. After successful surgery, the plastic cannulae cracked or broke, often due to striking fences or trees, and were not satisfactory. A thin, seamless steel cannula was designed (Figure 1) which has proved to be successful in collecting samples of forage grazed by dairy cows. Modification of the surgical procedure as reported by Cook *et al.* (1) was followed for the installation of the steel cannula in the esophageal fistula. This is as follows: The animal is most conveniently anesthetized with a general anesthesia (equithesin), then placed in right lateral recumbency and restrained by confinement of all limbs. A padded neck support 12 in. by 12 in. is placed under the middle third of the neck. This facilitates the operating procedure, bowing the neck and making the esophagus more accessible. The ventral cervical region is clipped free of hair thoroughly and disinfected. A ventral skin incision approximately 6 in. in length is made in the middle one-third of the cervical region. This incision is carefully extended through the subcutaneous fascia, thence bluntly extended between the medial margin of the sterno-cephalicus muscle and the lateral margin of the omohyoideus muscle. Care is taken to avoid the common carotid artery and vagosympathetic nerve trunk present at the right dorsal quadrant of the trachea.

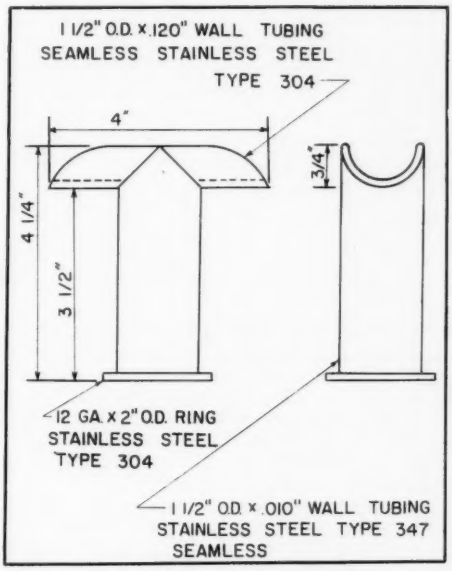

FIG. 1. Stainless steel cannula.

The esophagus is isolated by blunt dissection and exposed to view. Identity of the esophagus may be enhanced by the passage of a large stomach tube. The esophagus is packed off from the surrounding tissue by sterile towels or sterile sponges. An incision approximately 3 in. in length is made longitudinally through the esophageal wall into the lumen.

The flanged end of the fistula cannula is inserted into the lumen of the esophagus. The anterior and posterior limits of the incision are closed by inverting the edges of the esophageal submucosa with 0 chromic catgut sutures. Next, a purse-string suture is employed to hold the submucosa of the esophagus around the tubular portion of the fistula, using a continuous pattern of No. 3 medium-chromic catgut, and the subcutaneous fascia is sutured using the same pattern and suture material. It is advisable to suture the subcutaneous fascia, thus aiding in the support of the tubular portion of the cannula. The skin incision is closed in a conventional manner. After-care consists of antibiotic and supportive therapy.

Before the installation of the cannula, the

[1] Preliminary report presented at Southern Division, American Dairy Science Association, Annual Meeting, February, 1961, Jackson, Mississippi.

animal is adjusted to a pelleted feed of ¼ in. in diameter, commonly referred to as rabbit- or poultry-sized pellets. These pellets are fed 2 wk. prior to the operation and 1.5 wk. following the operation, after which the animal is adjusted to alfalfa hay gradually for a few days. At the end of 2 wk. following the operation, the animal can be used in experimental trials to collect samples of forage. A large canvas bag directly under the cannula permits accumulation of the sample. A No. 5 rubber stopper is placed in the open end of the cannula for closure. Animals with a steel esophageal fistula cannula have been used in research for 2 yr.

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RUMEN CONCENTRATIONS OF VOLATILE FATTY ACIDS AS AFFECTED BY FEEDING FREQUENCY

Increased daily weight gains by dairy heifers have been observed when the same quantity of feed is divided into small portions and fed several times a day (7, 9) and a similar response was obtained in sheep (5). On the other hand, Gibbons (4) found no difference in weight gains when he compared two feeding frequencies on pairs of monozygous twin heifers.

The milk production of a group of first-calf heifers did not respond to an increased frequency of feeding (7). Rakes *et al.* (9) suggest that the difference between heifers and cows may be due to a different optimum ratio of volatile fatty acid (VFA) production for maximum growth or fattening and for maximum milk production.

The present study was initiated to determine whether rumen concentrations of volatile fatty acids were more consistent throughout the day when animals were fed eight times a day as compared to twice-daily feeding. In addition, it was thought that the differences in the ratio of acids might provide explanations for the previously observed increases in rate of gain resulting from more frequent feeding.

Two fistulated Brown Swiss animals served as experimental subjects. One was a 1,220-lb. steer, and the other was a 990-lb. unbred female. They were housed in box stalls with access to outdoor exercise areas. Water and a mineral mix was provided ad libitum, except during experimental periods when the daily consumption was recorded. During and prior to the experimental period, each animal received a maintenance ration composed of 16 lb. of long alfalfa hay and 2 lb. rolled barley, which approximately met Morrison's maintenance requirements for total digestible nutrients (8). The exact amount of feed consumed during the experimental period was fed five to seven days before the experimental period.

On the two-times-per-day feeding regimen each animal received 8 lb. of hay and 1 lb. of barley at 5 A.M. and 5 P.M. When fed eight times per day, each animal was given 2 lb. of hay and 0.25 lb. of barley at 12, 3, 6, 9 A.M. and 12, 3, 6, 9 P.M. The experiment was conducted once on each feeding plan, and both animals were on the same plan during each of the two experimental periods.

During the experimental period, rumen fluid samples were taken on the hour for 24 consecutive hours. Samples were immediately taken to the laboratory, strained through 100-mesh cheese cloth, and the fluid centrifuged for 10 min. at about 5,000 r.p.m. Samples were then acidified with 50% sulfuric acid at 3% of the fluid volume. Samples were either analyzed immediately or stored at 0 °C. until analyses could be performed.

The VFA concentration of the rumen fluid was determined by the column chromatography method of Keeney (6), with modifications suggested by Vidacs and Knox (10). With each set of determinations consisting of ten columns, a standard containing 60% acetic acid, 25% propionic acid, and 15% butyric acid was added to one column. If analysis of the standard did not correspond closely to the calculated percentages and concentration, the entire series was repeated.

The data clearly indicate that feeding frequency influenced the total concentration of VFA, the percentage concentration of individual VFA, and the ratios of VFA. The mean values for total VFA concentration on the two-times and eight-times levels were 105.9 ± 7.8 m*M* and 114.8 ± 4.0 m*M* of acid per liter of rumen fluid. Although the response was very similar in each animal, the heifer consistently maintained a higher concentration of VFA than did the steer. The increase in total VFA concentration was significant when the feeding frequency was increased from two to eight times per day as determined by the "t" test ($P = < 0.01$).

As shown in Figure 1 there were two different patterns of acid concentrations. The curve de-

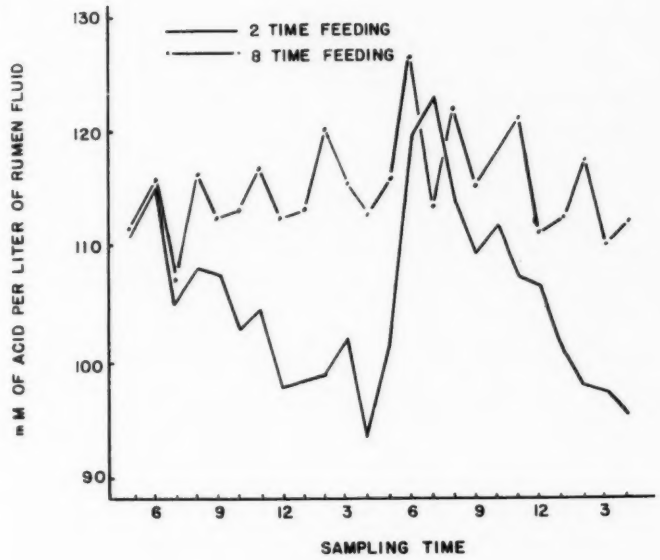

FIG. 1. Variations in the total concentration of volatile fatty acids over a 24-hr. period on two-times and eight-times-per-day feeding.

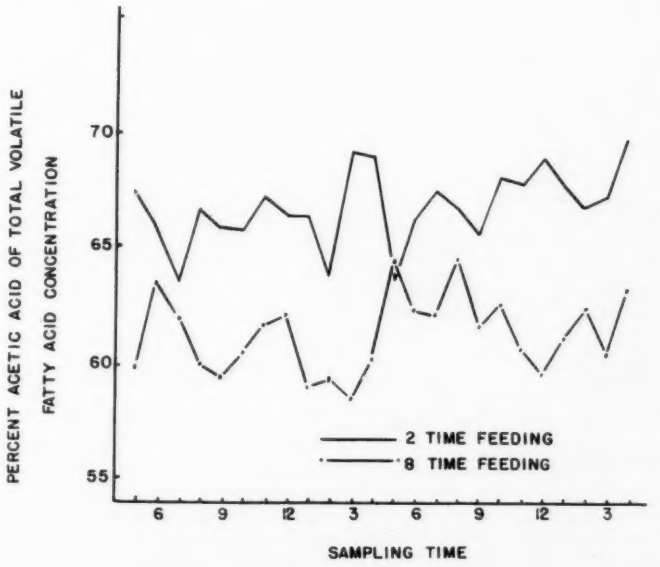

FIG. 2. Acetic acid as a percentage of the total volatile fatty acid concentration over a 24-hr. period on two-times and eight-times-per-day feeding.

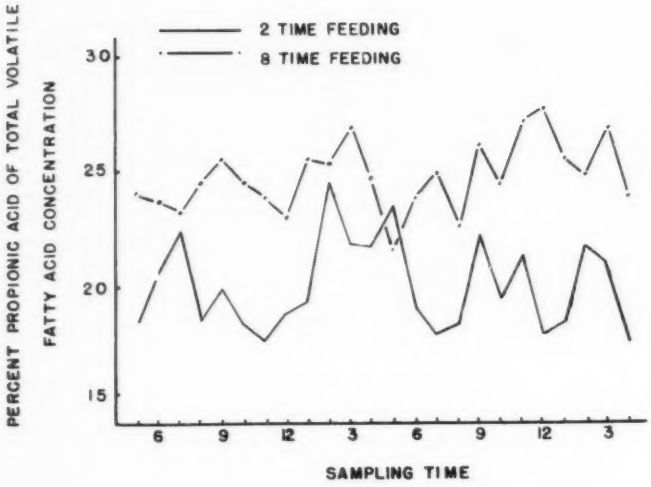

FIG. 3. Propionic acid as a percentage of the total volatile fatty acid concentration over a 24-hr. period on two-times and eight-times-per-day feeding.

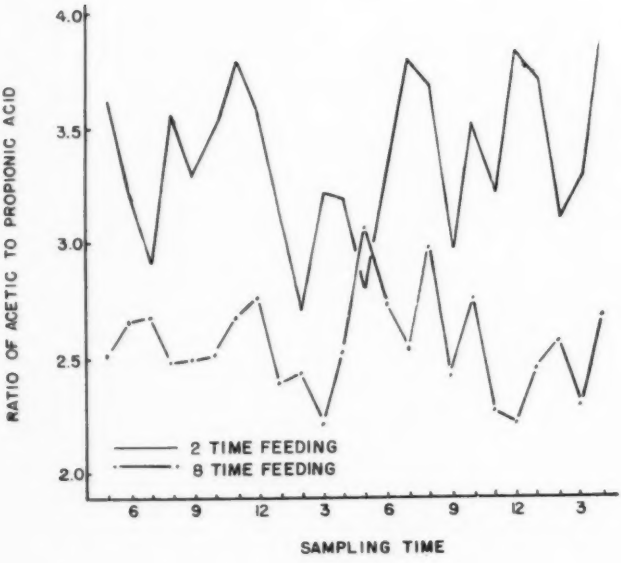

FIG. 4. Ratio of acetic to propionic acid over a 24-hr. period on two-times and eight-times-per-day feeding.

picting VFA concentration on the two-times feeding showed two distinct peaks both within 1 to 2 hr. after feeding, which dropped to the lowest point the hour before the next feeding. This two-peaked curve was in contrast to that obtained from the eight-times feeding trial, where a more constant concentration of VFA is indicated during the 24-hr. period.

Acetic acid as a percentage of the total concentration was consistently higher on the two-times than on the eight-times feeding (Figure 2). The opposite was found in propionic acid, which showed a higher percentage on the eight-times feeding (Figure 3).[1] Butyric acid concentrations followed the same trend, although the differences were smaller and more variable. In each case, the difference in level between feeding frequencies was statistically significant ($P = < 0.01$).

The mean ratio of acetic to propionic acid (A/P) was higher on the two-times than on the eight-times feeding regimen (3.49 ± 0.34 vs. 2.55 ± 0.24). The same was also true for the acetic to butyric acid (A/B) ratio (5.05 ± 0.74 vs. 4.14 ± 0.22). Likewise, the ratio of propionic to butyric acid (P/B) was found to be slightly higher on the eight-times than on the two-times feeding (1.64 ± 0.14 vs. 1.54 ± 0.30). Since there was more variation in the P/B than either the A/P or A/B ratio, and the difference was small, it is assumed that there was little, if any, real difference between the two feeding systems with respect to the P/B ratio.

The lower A/P ratios found with more frequent feeding may provide a clue to the greater gains resulting from this practice, for it has been shown (1) that acetate is utilized less efficiently than the other VFA, at least when acetate is supplied in high proportion to other acids.

Additional evidence for the involvement of acid ratios is provided by the work of Elliott and Loosli (2), who have shown that the A/P ratio is closely related to the efficiency with which digestible energy is converted to 4% fat-corrected milk. They found a low A/P ratio to be associated with higher milk production.

A lower mean A/P ratio was associated with eight-times-a-day feeding in the present study, which might contribute to more efficient energy utilization if the values obtained for lactating cows can be extrapolated to animals on a maintenance diet. Ensor *et al.* (3) obtained greater weight gains with a lower A/P ratio, but had less efficient production of 4% FCM on this type of ration.

Another possible explanation for differences in rate of gain is related to the total concentration of VFA. On the eight-time feeding it was found that there was a significantly higher concentration of VFA than on the two-times feeding, and this difference became more pronounced with time after feeding. The two peaks of VFA concentration observed on the two-times feeding (Figure 1) may increase total acids, principally acetic, at two periods during the day, which might lower the efficiency of the VFA utilization by the host. The A/P ratio obtained in neither the two-times nor the eight-times feeding procedure showed any relation to the peaks of total VFA concentration or time after feeding. The lower weight gains on a two-times feeding procedure may be related to the pattern of total acid concentration throughout the day.

The results presented here, together with the observations on the relation of the A/P ratio to milk production (2), would suggest that more frequent feeding would result in higher milk production. However, Mochrie *et al.* (7) found no advantage in milk production from more frequent feeding of heifers.

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[1] Percentages expressed in Figures 2 and 3 are by weight.

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OUR INDUSTRY TODAY

AN INQUIRING LOOK AT ANTIBIOTIC RESIDUE CONTROL[1]

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Antibiotic residues in market milk were a real live subject a year ago, but the furor about antibiotic residues in milk has quieted down a little now and this may be good or bad. It means either that nothing is being done about antibiotic control or that certain objectives have been attained. The achievements of the dairy industry will be briefly reviewed, but actually the main theme is less concerned with achievements than with the new problems arising from our attempts to rid milk of antibiotic residues.

Practical means to control the antibiotic menace. The only practical way to institute residue control for antibiotics is to institute a solid test program. Once milk producers are aware of the existence of such a program, it serves as a psychological deterrent to their breaking the regulations. Testing for antibiotics is one of the most inexpensive programs of the various test programs conducted by the dairy industry, as such tests cost only a few cents each.

Once-a-month testing among producers is generally sufficient, but the testing must be regular and good records must be maintained. The State of Pennsylvania now has a law requiring the testing of all producer milks once a month. A positive antibiotic test automatically excludes the producer for two days. Another state, Wisconsin, is considering a similar law.

Education should follow up closely positive test results in the field, for at this point education, usually through personal remarks by the fieldman, becomes most effective. All positives should first be confirmed by penicillinase or by heating techniques, and regular offenders should be given the full penalty previously decided upon. Simple tests based on the disc assay principle or on test tube activity outlined in the 11th edition of the American Public Health Handbook of Standard Methods (1) are the most suitable at the grass-roots producer level. But for those public health officials checking bulk tanks and concerned with the strictest interpretation of present-day regulations, the best test, because of its greater sensitivity, is the *Sarcina lutea* disc assay test conducted at 25° C. for 18 hr. (4).

Current achievements. Since January, 1960, the efforts to bring antibiotic residues under control have gained much momentum. Now, almost a year later, some accounting of this nation-wide effort is possible from recent surveys made by Cornell University (Table 1).

[1] Portions of this manuscript were presented at the Ohio State University Dairy Conference, Columbus, Ohio, 1961, and at the New York State Sanitarians' Meeting, Syracuse, New York, 1960.

Most of the significant results are summarized in Tables 1 and 1a. A nation-wide average incidence of 6.0% has dropped to 0.54% incidence. This is a remarkable reduction and made possible only through the full cooperation of all parties concerned.

Some highlights garnered from this study are of interest. Dairy industrial laboratories rather than state units carried out most of the testing, and this is as it should be. The most widely used test by the cooperating laboratories wos the *Bacillus subtilis* disc assay test as described in the 10th edition of the Handbook of Standard Methods of the American Public Health Association.

The incidence reported by state health and agricultural laboratories was 0.44% and that by the industrial dairy laboratories was 0.56%. This is a good check, giving substance to the

TABLE 1

The national incidence of antibiotic residues in producer milks

Organizations reporting	Number of producer milks analyzed	Number positive for antibiotics	Incidence
(January 1 to October 15, 1960)			
17 dairies throughout the United States	655,763	3,640	0.56
28 State Health and State Agriculture Departments throughout the United States Total	112,705	493	0.44
45 Cooperating organizations	768,468	4,133	0.54

TABLE 1a

The New York State incidence of antibiotic residues in producer milks

Number of producer milks tested	Number positive for antibiotics	Incidence (%)
(January 1 to October 15, 1960)		
129,214	568	0.44

validity of these data. State laboratories perhaps exercised greater care in guarding against false positives, thus leading to a lower incidence.

Improvement, then, in our antibiotic residue problem is marked. Yet, from a public health point of view, does this lowered antibiotic incidence mean our troubles are over and that, in general, the problems that remain are minor? The answer is No—on both counts. A consideration of the more significant is presented.

False positives in analytical testing. Natural inhibitory substances of milk inevitably rear up to bedevil the unwary. Differing from blood, for which early tests were designed, milk shows natural inhibitory substances in greater profusion. These give zones on disc assay which have no relation to pharmaceutical antibiotics.

The problem of false positives is not an imaginary one and the closer one gets to the milk of the individual producer's cows, the more the likelihood exists of encountering false positives. Also, raw milks with certain strains of bacteria or with total high counts are more prone to show false positives on disc assay. A number of such instances are cited in the scientific literature (5-7).

False positives result from a variety of causes, but their appearance is unpredictable. It is possible to run a number of weeks without spotting any, but then suddenly a few may appear. Heating to 180° F. 2 to 5 min. invariably eliminates all of these false positives without materially affecting the pharmaceutical antibiotics that might reside in that milk.

The existence of even one false positive among 10,000 producer samples does a grave injustice to some individuals if no heating or other confirmatory check is applied. This is because that zone must be taken as bona fide evidence of the presence of either penicillin or some other pharmaceutical antibiotic. Without heating and the use of penicillinase there is no scientific means to fully characterize the positive zone other than penicillin. The Federal laws indict all pharmaceutical antibiotics, and not simply penicillin, as milk adulterants, so that penicillinase alone is not sufficient.

Penicillinase in milk. The Food and Drug Administration is cognizant of the remote possibility that milk producers may be using the enzyme, penicillinase, to destroy penicillin residues in milk. The addition of penicillinase is an adulteration and contrary to law.

It is an extremely easy task to check milk supplies for the enzyme, penicillinase, by simply reversing the procedure for detecting penicillin. To the milk in question a small amount of pure penicillin (0.1 I.U/ml) is added and then the disc assay test is applied. The presence of penicillinase is indicated by the absence of a clear zone.

Transfer of penicillin and its significance. Reported transfer of penicillin in cows has created much confusion. Transfer means that penicillin infused in one quarter of an animal may travel, by udder tissue or blood, into adjoining untreated quarters. If large amounts transfer, a milk producer may unknowingly ship milk to market containing detectable amounts of penicillin.

Dairy scientists have reported since 1951 that penicillin transfer does not occur readily, but a number of field workers are under the impression that massive transfer of penicillin is a common occurrence with many cows. Because of the availability of more sensitive analytical methods, a certain justification exists for re-examining this subject.

Dr. Blobel of the University of Wisconsin, in a recent excellent study, reported that 14 of 17 cows inserted with 100,000 I.U. penicillin showed transfer (2). His method of analysis, however, was the ultrasensitive *Sarcina lutea* test and not the standard disc assay test. Even by this method, the amounts transferred were exceedingly small, 0.005-0.01 I.U., and were detectable for only about 24 hr.

At Cornell University, in a recently completed cooperative study between the College of Agriculture and the College of Veterinary Medicine (3), an investigation on 50 cows revealed only two cows transferring penicillin, and these were at very low levels (0.01 I.U.) lasting only two milkings. In both of these large-scale experiments, if the standard disc assay test with 0.05 I.U. sensitivity were used, no detection of penicillin would have been possible.

Most scientific evidence to date indicates that transfer of penicillin in terms of concentration and persistence is not a problem of serious proportions with our present system of testing.

A double standard of comparison. A more serious aspect of antibiotic contamination in milk has been overlooked, however. This aspect is readily apparent by anyone familiar with the significant phases of antibiotic control. It is illustrated by the fact that on the identical 17 cows studied by Blobel and with the ultrasensitive *Sarcina lutea* method, the penicillin in milk from the treated quarters persisted up to 120 hr. Each quarter was treated with 100,000 I.U. penicillin G, crystalline, water-in-oil. It is the penicillin preparation most widely used and considered by many to be the most effective against mastitis.

Why didn't it clear up in 72 hr.? The simple reason is that in applying a more sensitive analytical method to show up transfer, it was possible now to observe the traces of penicillin persisting in milk from the treated quarter for long periods. According to FDA regulations, evidence of such long-time retention would rule out the use of this penicillin preparation, because 96 hr. of retention is the limit allowed any preparation. Most present effective penicillin preparations, particularly ointments, fall in this category if an ultra-sensitive method is used at the individual cow level. Perhaps

such long-lasting drugs can be placed under prescription rules.

Factors observed in the recent Cornell study (4), which contributed antibiotic residues to fluid milk, in order of importance were: (1) persistence of commercial drugs in treated quarters well after 72 hr.; (2) careless contamination of milk from untreated cows standing next to treated cows; (3) intramuscular injections, and (4) transfer. The latter two factors were relatively minor in importance. It was calculated that about 40 times more antibiotic found its way into milk for consumption through persistence in treated quarters than from transfer.

Extension recommendations for antibiotic residues and their meaning. Recommendation differences between extension departments of state universities are readily apparent (Table 2). Some extension services simply recommend that milk be withheld only for 72 hr. after treatment; others require that milk should be retained from the cow for eight days, or 16 milkings. Twenty-two extension services recommend rejecting milk from the entire cow while 11 others recommend holding out milk from the treated quarters.

It is interesting to point out that between states which recommended rejection from the treated quarter, and those recommending rejection of milk from the entire cow, the rate of incidence after 1 yr. was similar.

There are some who feel that all milk from a treated cow should be withheld for 72 hr., even if only one quarter has been treated. This is despite the fact that the milk from the untreated quarters may be normal in every respect and that by this practice a heavy loss of such normal milk will ensue. It is based on the laudable principle that no milk from a diseased cow should be included in the milk supply.

Unfortunately, from the point of view of objectivity, even among highly qualified veterinarians and other public health officials, there is no unanimity of agreement as to the definition of a diseased cow. Most early recommendations for antibiotics were to withhold milk from the treated quarter only. The later trend to withhold milk from the entire cow resulted more from fears of possible transfer of penicillin through quarters than from the consequences of using milk from diseased cows. Actually, with the present system of withholding milk from the entire cow, there is no guarantee that milks from diseased cows are not reaching the consumer. Treatment with antibiotics and withholding all milk for 72 hr. may satisfy the legal rulings, but if the antibiotics are ineffective, this milk still may be coming from diseased cows unless good checking is conducted by the producer or other qualified persons.

Professional groups, such as the American Veterinary Medical Society, are formulating their own recommendations. Differences have arisen because many believe present Federal recommendations are incomplete and are not based on the best information leading to antibiotic-free milk. Poorly defined and impractical recommendations between states can undermine the confidence of the cooperating milk producer in any antibiotic-control program, seriously affect him economically by unnecessarily heavy loss of milk, and all this without influencing the welfare of the consumer.

Incompatibility of zero tolerance. The Food and Drug Administration insists on the principle of zero tolerance for antibiotics in milk. No one, least of all this writer, questions the desirability of such a ruling if it is necessary to protect the consumer. But in practice, it is becoming apparent that a strict insistence on zero tolerance is incompatible with present

TABLE 2

Recommendations for antibiotic residue control by state extension services in the United States

(As of October 5, 1960)

A. How long should milk be withheld after treatment with antibiotic intramammary infusion?

No general recommendation	For 72 hr.	For 96 hr.	For three days or six milkings	For six to eight days water-in-oil or oil
6	34	1	3	2

B. Milk should be held from entire treated cow or treated quarters only.

Recommendation not clear	All milk from treated cows	Milk from treated quarters only
1	24	12

C. How long after intramuscular injection of antibiotics should milk from the entire cow be withheld?

No recommendation	For 72 hr.	Three to five days	Five days	Six days	Seven days
22	7	2	8	1	3

D. Should milk be withheld after feeding cow feed containing antibiotics?

Yes, for 72 hr.	No specific recommendation	Antibiotic feeds should not be allowed
1	37	1

Food and Drug and most university recommendations on the use of antibiotics for cattle disease. Methods of analysis now available, but not widely used, have become so sensitive as to make the 72-hr. limitation on most antibiotic preparations obsolete. Other more sensitive methods are bound to follow, making detection of traces much easier and longer than now possible. This is the ultimate course of zero tolerance. It has no ending.

Strict adherence to a policy of zero tolerance for antibiotics in milk can only lead to the ultimate abandonment of antibiotics as a therapy measure for cattle disease, because most antibiotic preparations, even today, can not satisfy the legal requirements. On the other hand, if interested workers and health officials would accept the limiting sensitivity of 0.05 I.U. penicillin per milliliter as the practical minimum level for enforcement and for extension recommendations, most of the problems would be solved. Inasmuch as the Food and Drug Administration personnel use tests in the field with this sensitivity, there is every reason to believe that they consider it as the practical cut-off point for action.

Public health aspects. The public health menace of minute traces of antibiotics in milk, in this writer's opinion, has never been studied on a truly scientific basis. The skimpy research reports on this subject concern themselves with only a few cases and generally lack proper controls. If the health problem is so acute, why in past years have no large, well-controlled experiments been conducted in hospitals under our numerous public health research grants? Why have health departments generally been reluctant to assign personnel and proper facilities for extensive checking of violators, or why have so few violators been legally penalized? And what has happened to milk with known detectable amounts of penicillin? Into what channels was it directed?

These questions are raised because, if a public health problem does exist, all the means at our disposal must be used to prevent even slightly contaminated milk from reaching the consumer. Present unconfirmed medical evidence states that the smallest traces still found in milk are 50 to 100 times greater than the sensitive human can tolerate, but if it can be scientifically proved that no public health problem exists at our present new low levels, then recognition must be made of this fact, because otherwise the broad plans to eradicate diseases in cattle are threatened, a result which too may affect public health. The future of antibiotic therapy for cattle diseases is dependent upon a wise interpretation of the consequences of minute traces of antibiotics in milk and subsequent realistic control recommendations.

SUMMARY

The great differences in recommendations between extension services about retention of milk from treated cows; the lack of good data on the effect of minute concentrations of antibiotic residues on public health, and the inconsistency of federal laws which insist on zero tolerance but which have not been able to eliminate all commercial drugs leading to infractions of zero tolerance principles are all there for us to ponder.

A solution to these problems, perhaps, lies in the bringing together of the divergent groups, followed by the formulation of uniform and realistic control proposals that are acceptable to the dairy industry and to regulatory groups.

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ASSOCIATION AFFAIRS

56TH ANNUAL MEETING, AMERICAN DAIRY SCIENCE ASSOCIATION, UNIVERSITY OF WISCONSIN, MADISON, JUNE 11-14, 1961

R. E. HODGSON, President and Presiding
Animal Husbandry Research Branch, USDA, Beltsville, Maryland

The 56th Annual Meeting of the Association was held in the heart of Dairyland at the beautiful campus of the University of Wisconsin on the shores of scenic Lake Mendota. The various meetings were permeated with a spirit of dedication to Dairy Science, which was inspired on this campus during the last century by the brilliant work of S. M. Babcock, H. L. Russell, and F. W. Woll, and further stimulated over the years by E. H. Farrington, W. D. Frost, E. B. Hart, E. G. Hastings, H. C. Jackson, and H. H. Sommer.

An informal get-together was held Sunday evening in the Elm Drive Commons, where members, their families, and friends enjoyed an annual reunion and refreshments.

This annual meeting began with a general program on the morning of June 12 in the Wisconsin Union Theater, with Dr. E. E. Heizer of the Department of Dairy Husbandry, presiding. The address of welcome was given by R. F. Froker, Dean of the College of Agriculture. The guest speaker was C. A. Elvehjem, President of the University. His subject was, Some Significant Dates in the History of Dairy Research at the University of Wisconsin. The morning's program included a symposium on Changes Which May Be Forced upon Us by Trends in College Enrollments. The participants were Dean A. E. Harlow of Oklahoma State University, Dr. B. L. Herrington of Cornell University, and Dr. George Hyatt of North Carolina State College.[1] Dr. R. E. Hodgson gave his presidential address at this morning's session.

A joint meeting of all sections was held on the morning of June 14 in the Agricultural Hall Auditorium. Dr. H. E. O. Heinemann of the Pet Milk Company spoke on the topic, Field Problems with Pesticide Residues.[2] Dr. H. E. Casida of the Department of Entomology was the discussant. This was an excellent program.

[1] It is hoped that the three papers presented at this symposium may be published in the JOURNAL OF DAIRY SCIENCE.

[2] This paper will be published in the JOURNAL OF DAIRY SCIENCE.

Speakers at the opening session: Dr. E. E Heizer, Chairman of the Department of Dairy Husbandry, University of Wisconsin; Dr. R. E. Hodgson, Director of the Animal Husbandry Research Division, USDA; Dr. A. E. Darlow, Vice-President and Dean of Agriculture, Oklahoma State University; Dr. Conrad Elvehjem, President of the University of Wisconsin; and Dr. R. K. Froker, Dean of the College of Agriculture, University of Wisconsin.

PRESIDENTIAL ADDRESS

Mr. Chairman, President Elvehjem, Guests, Ladies and Gentlemen—

We are gathered together for the 56th time to conduct the affairs of this Association. On July 17, 1906, a group of dairy educators met on the campus of the University of Illinois and organized what has become the American Dairy Science Association. These were wise men indeed, for the seed they planted grew through the years into this vitally important scientific and educational dairy organization.

Only two of the charter members are living today. We are most privileged to have one of them with us in the person of the venerable Dr. E. S. Guthrie. Dr. Guthrie was President of the Association in 1940. Dr. Guthrie, we are proud of your many accomplishments and are very happy that you are here.

At the first meeting in 1906, E. S. Guthrie, then of The Ohio State University, described his experiences in herd improvement and sanitary milk production. Dr. Guthrie was born in Iowa in 1880 and had just completed his B.S.A. degree at Iowa State the year before this first meeting. He served at Ohio until 1908, when he joined the staff at Cornell University. There he earned an M.S. degree in 1910 and a Ph.D. degree in 1913 in Bacteriology. Though he became Professor Emeritus in 1948, he continues to be active in research in his favorite field of flavors in milk. He participates regularly in the annual meetings of the Association he helped organize. He has given generously of his time to this organization during the past 50 years. He has been an outstanding teacher and a productive research worker during his career, having published many articles and a textbook on butter.

Dr. E. S. Guthrie, emeritus Professor of Dairy Industry from Cornell University, and a charter member of the American Dairy Science Association, discusses the Association meetings with Dr. N. F. Olson of the Department of Dairy and Food Industries at the University of Wisconsin.

The other living charter member is C. F. Doane, who presently resides at Salem, Oregon. Though suffering the infirmities of a light stroke, Mr. Doane writes in part:

"It would, of course, be a great privilege to meet with the Association on the campus of my old school at Madison. The years and the miles are too great, but in spirit I will be there, mingling with the crowds, listening to the papers and the conversation which will be far above my head, for Ph.D.'s were the exception with that first group and not the rule. With you I will look again at beautiful Lake Mendota and perhaps take a late stroll over the campus and look again for the lights burning in old South Hall, where Dr. Babcock toiled far into the small hours. And, at the end, make a sincere wish—or might we call it a prayer—for the continued growth and influence of the Association in which I had a very small part over half a century ago."

What a wonderful message.

Now, it is my privilege to introduce to you the officers of your Association:

Dr. E. L. Jack, Vice-President, University of California, Davis, who at the close of this meeting will become your new President.

Dr. A. C. Fay, Past President, North Miami, Florida. Absent because of illness.

Dr. N. P. Ralston, Director, Michigan State University, East Lansing.

Dr. G. H. Wise, Director, North Carolina State College of Agriculture, Raleigh.

Prof. F. J. Doan, Director, also Chairman of the Journal Management Committee, Pennsylvania State University, University Park.

Dr. J. T. Reid, Director, Cornell University, Ithaca, New York.

Prof. E. C. Scheidenhelm, Director, University of Kentucky, Lexington.

Dr. H. F. Judkins, the Venerable Secretary-Treasurer, White Plains, New York.

Dr. E. O. Herreid, the hard-working, efficient Editor-in-Chief, University of Illinois, Champaign.

Dr. George W. Trimberger, who has done an outstanding job this year as Chairman of the Program Committee, Cornell University, Ithaca, New York.

Lewis D. Meyer, Chairman of the Student Affiliate Branch, Michigan State University, East Lansing.

To each member, his family, and our guests from the United States, Canada, and other foreign countries, welcome to the 56th annual meeting of the American Dairy Science Association. We are privileged to have this gathering on the beautiful campus of the University of Wisconsin. One-seventh of the nation's milk supply is produced in this State—the heart of America's dairyland. It is fitting that we meet here during the month of June as America celebrates June Dairy Month.

On behalf of the members of the American Dairy Science Association, our sincere thanks

to you, President C. A. Elvehjem, and the officials of the University for inviting us here. It is our privilege to use these fine facilities. We are your guests, and we deeply appreciate the hospitality that is so clearly evident. The Host Committee for this meeting is under the leadership of Dr. L. H. Schultz, whom I present to you at this time. Dr. Schultz and many other individuals from the different Departments working on the several committees have labored hard to make our stay enjoyable, fruitful, and rewarding. To them, our special thanks. Appreciation also is extended to the organization and individuals of the State of Wisconsin and elsewhere who have cooperated with these folk in organizing this meeting.

This annual meeting is particularly significant to me personally. This is my Alma Mater, and my introduction to the great dairy industry began on a farm only a few miles from here. I am grateful to the members of this Association for calling on me to serve as your President. It has been a rewarding experience and I only hope that I have in good measure met your expectations.

The constitution states that the name of this organization shall be the American Dairy Science Association. The objective "is to promote the welfare of the dairy industry by stimulating scientific research, improving educational methods, encouraging worthy intra-industry and inter-industry cooperative endeavor, and by publishing the JOURNAL OF DAIRY SCIENCE and other official periodicals." This is a worthy objective. Through the 56 years of its existence great advances in the dairy industry have taken place. The members of this Association have contributed much in a great variety of ways to this advancement. The Association itself has grown from a membership of 17 the first year to over 2,600, plus some 600 student affiliates in 1960. Almost 5,000 copies of the JOURNAL OF DAIRY SCIENCE were issued monthly during this year to the members and some 1,700 subscribers. The JOURNAL is now being sent to 65 different countries.

The American Dairy Science Association is a professional society of dairy workers. It is one of many such societies organized voluntarily by individuals interested in a particular field of work, joining together to promote their professional advancement as well as to exert favorable influence in the area of their interest. Professional societies are unique institutions in this great free land. They are entirely free of governmental and political influences and can function of their own free will, being guided only by the members. These societies develop as their main activity the publishing of technical journals, thus providing an avenue for the issuance of the results of their research and related activities. In years past, and even at present, this seems necessary, since there are limited outlets elsewhere for such publications. The Society-published scientific journals are supported out of membership dues, advertising, and the sale of reprints.

Our own fine JOURNAL OF DAIRY SCIENCE each month is filled from cover to cover with articles coming from public-supported research institutions. No place else can you find more information on dairy science and this JOURNAL is the world's foremost source of this kind of information.

Societies provide additional means for professional advancement through their annual meetings. As is the case at this meeting, we come together to present a series of papers giving the latest results of our research and related activities carried on at our home institutions. We are here to exchange ideas, to be brought up to date, to learn what the other fellow is doing and what he knows, to compare notes on extension and teaching methods, perhaps to make a contact for a better job, to single out and honor those few who have made outstanding achievements, and—not the least—to have a good time. We study problems and pass resolutions that we believe will favorably influence trends and actions for the over-all betterment of our Association and of the industry that we serve.

The American Dairy Science Association's membership and program are intimately interwoven with America's great system of land-grant colleges and universities and with the United States Department of Agriculture, both of which were created by the Morrill Act, passed by Congress, and signed by President Abraham Lincoln in 1862. If you are a member of one of these institutions, it provides you with your salary and the material things with which you work. If you are not, no doubt you are a graduate of one of the colleges or universities and your loyalty to it remains firm. The great system of federal and state research, education, and extension has grown out of this vital piece of legislation, sponsored and acted on by wise men a century ago. This legislation has made possible in a very large measure the information, talent, and techniques that have enabled American agriculture, and particularly the dairy industry, to develop and advance. It is significant that next year America will recognize and celebrate the centennial of Agriculture. The annual meeting of this Association, at the University of Maryland in 1962, will make proper recognition of the centennial.

The American Dairy Science Association is dedicated to the service of the dairy industry. But how can we be of even greater service? Our Association does not control the resources that go into the research, education, and extension programs that concern us. It can not gauge directly the activities of industrial dairy companies, even though many of you are members of them.

This Association, through its membership, can, however, exert great influence on activities of public and private-supported institutions,

because in this Association is gathered together the technical and professional knowledge that has been, and will continue to be, required by the dairy industry to enable it to advance efficiently and keep abreast of the times. By precept and example, by study, consultation, information, resolution, influence, and guidance this Association can assist and guide those institutions and their programs working in all aspects of the dairy industry. This is, it seems to me, the role of the American Dairy Science Association in the complex structure that makes up the great American dairy industry.

To do these things in an outstanding manner, the Association and its members must know the important problems, the trends, and the many other things that affect dairying, and we must know how to arrive at solutions. The approach will be made through research, education, and extension in all the facets of production, processing, utilization, and marketing of milk and its products.

What are some of the problems that concern the dairy industry? Only a few of the important ones can be touched on here. Of first importance, I think, is the declining acceptance of milk and milk products by American consumers. The per capita production of milk has declined from 832 lb. in 1946 to 683 lb. in 1960. The per capita utilization of milk in all dairy foods has declined from 786 lb. in 1946 to 670 lb. in 1960. Per capita consumption of fluid milk and cream has declined from 389 lb. in 1946 to 338 lb. in 1960. The United States stands 15th among the nations of the world in the per capita use of dairy foods. One might ask why this has happened in America, where we have the world's highest standard of living, the world's greatest consumer purchasing power, one of the best milk supplies from the standpoint of healthfulness and safety. During this period there has been a steady increase in the use of solids-not-fat and a decrease in the use of milk fat. The competition of other food fats has accounted for much, but not all, of this decline in the use of milk fat. This is a fact and the dairy industry must meet this competition. Another factor, of course, is the so-called health scare of animal fats. This is a significant problem that needs a great deal of research to arrive at the true facts.

In the next 50 years our population will double and our food needs will nearly double. Will the dairy industry enjoy its share of this increase in food needs, and, if so, will it be able to meet its obligations? The answer to these questions will depend greatly on research and education and on public understanding and confidence that dairy foods in good measure are needed in our diets and that these foods are economical and safe. Surely there is a wide range of questions on this problem—from breeding cows with a different composition of milk to the development of new dairy foods and to the role of milk fat in circulatory diseases. These questions are crying for the attention of all segments of our membership and others in the dairy and related industries. Real leadership is needed here.

The second problem area is the relative inefficiency in the production of milk on American dairy farms. I know we have made good progress through the years. Milk production per cow has increased 43% from 1946 to 1960. Yet the feed units consumed per 100 lb. of milk produced have remained essentially unchanged. We are feeding more and better feeds, but we have not broken the barrier to increase feed conversion efficiency.

We have many fine herds of high-producing cows, but not enough. We have been improving the genetic ability of our cattle steadily but do we really know how good our cows are? Let me cite a few comparisons. The average milk production per cow in the United States in 1958 was 6,585 lb. For the United Kingdom it was 6,819 lb.; West Germany 7,070; Denmark 8,019; Belgium 8,282; and Netherlands 9,022 lb. All these countries have a good history of record-of-performance testing. In the United Kingdom, 28% of the cows were on production test, West Germany 32, Denmark 59, and Netherlands 66%. In contrast, in the United States, less than 15% of the dairy cows were on test. There is no question but that the high participation in record-of-performance activity in these other countries has provided good cattle, good feeding and management, has eliminated low-producing cows, and enabled a high annual output per cow. We would do well to follow their example with a much higher participation in this important activity.

This Association, in cooperation with the State Agricultural Extension Services and the U. S. Department of Agriculture, sponsors our National Dairy Herd Improvement program. Under this program three plans of recording are available, one of which will meet the needs of any dairy farmer. In addition, each of the Breed Registry Associations conducts recording programs. I believe a recording program is the key to successful dairy farming and that high production per cow is one thing that will keep the dairyman in business. Why is it that we are doing so poorly in this country with milk recording? This is a great challenge to our dairy production extension members.

Some say that increasing production per cow will only add to and aggravate price-depressing surpluses. But the overwhelming reason for high unit production is that it is efficient production, yielding greater returns over production costs. Supply and demand relationships are more realistically regulated by the number of production units involved. Indeed this is what has been taking place as indicated by the increases in production per cow, increases in size of herds, decreases in milk cow

numbers, and decrease in number of farms with milking cows.

There is tremendous opportunity for our production research, education, and extension members and those in the industries servicing and supplying milk producers to render service to dairy farmers. They need information that will help them improve the whole dairy farm production process from feed production, breeding, artificial insemination, feeding, disease control, and efficient management. These needs await your earnest efforts.

A third problem area I choose to mention is the wide spread between the price the farmer receives for milk and the price the consumer pays for milk and milk products. This involves both processing and marketing. Science and technology have done a wonderful job in developing new techniques of processing which have resulted in uniform high quality products with a long shelf life. Dairy foods are packaged and distributed and made available to consumers in a variety of easily accessible ways. The dairy industry has kept abreast with other food industries in this respect. All this fine service, I am sure, is appreciated by the consumers, but it has not resulted in selling more dairy foods on a per capita use basis. Is the cost of getting milk and dairy foods to consumers too high? How does the cost of processing and distribution of fluid milk, for example, in this country compare with that of some of our neighbors? Such a comparison is available in a recent FAO report for the years 1953-57.[1]

This report shows that for the Netherlands the average price for milk received by the producer was 3¢ per pound, the average price paid by the consumer averaged 3.5¢, and the per cent that the retail price was of the producer price was 117; for the United Kingdom the relationship was 4.4¢ for producer price, 6.4¢ for retail price, and 145% spread; for Denmark the relationship was 2.5¢ for producer price, 4.0¢ for retail price, and 160% spread; for New Zealand the relationship was 2.0¢ for producer price, 4.1¢ for retail price, and 205% spread; for Canada the relationship is 3.0¢ for producer price, 8.0 for retail price, and 267% spread; and for the United States, the relationship was 4.1¢ for producer price, 11.0¢ for retail price, and 268% spread.

It appears from this that except for our neighbor, Canada, other leading dairy countries are able to get fluid milk to consumers at a much lower markup than is the case in America. I do not suggest that we here should adopt the techniques used by other countries, and I recognize there are differences in costs. I do believe that this points out the great challenge that lies before our dairy scientists, technologists, marketing specialists, and others and the opportunities to improve the efficiency and lower the costs of processing and of getting milk and dairy foods to consumers. To this I would also add the challenge that exists for the continuing development of new kinds of dairy foods, new processes and techniques, and new packaging and marketing procedures to benefit this industry, and this does not mean punching artificial holes in Swiss Cheese.

The word science in the name of our Association symbolizes the deep interest we have in research as applied to all phases of the dairy industry. Many of you members are engaged in research. You are the information producers and the dairy industry has a great stake in you. Many of you are public servants and your efforts are supported by the public. It is your obligation to choose wisely the research you undertake and to perform your tasks efficiently. I believe you should be concerned more with basic research. It is from such work that understanding will come to solve the critical problems facing agriculture and the dairy industry. Each of us must be concerned with using our resources to the best advantage on the most important things and avoid duplication as much as possible. We must exhibit an awareness to our administrators that the research resources are not unlimited and that we as individual scientists are abreast of the times, that we have knowledge of the work needed and how it should be done. We must be willing to work in teams in problem areas, but at the same time we must also guard our individual freedom and right to self-expression in our specialized field.

In the area of extension education, the challenges and the opportunities for service are legion. The extension approach is rapidly changing, because the problems are changing. Adjustments in agriculture and dairy farming come rapidly and we are dealing with a much different clientele than was the case only two decades ago. The modern extension worker must not only be expertly versed in subject matter but also well versed in teaching methods and techniques. As extension workers we must be continually examining our teaching methods and the things we teach. We must sort out for inclusion in our program those subject matters that are really important. The extension worker must maintain closer contact with the researcher and be able to quickly sort out information that is useful in his program and get it to the farmer and into use quickly. He must also not overlook opportunity to bring to the investigator problems in the field that require research attention.

What I have said about the extension educator applies equally to the resident teacher. Subject matter included in the courses we teach must be under continual examination and up-dating. As teachers we must continually

[1] FAO Report No. 12, National Dairy Policies in Advanced Countries, FAO Policy Studies, Rome, 1960.

be asking ourselves: "Are we teaching the right things? Are we putting emphasis in the right places? As teachers of graduate students, are we giving a candidate enough opportunity to carry out research on his own in the basic fields of science, so that when he receives his degree he is really prepared to take up a research career on his own?"

It is my purpose to raise these points, because as individuals and as the American Dairy Science Association we are concerned and are desirous of doing something about them.

The American Dairy Science Association activities are organized under the elected officers making up the Executive Board and in sections of Extension, Manufacturing, and Production. In all, there are currently 41 recognized committees and seven association representatives with a total of over 300 members working on specific tasks. In addition, the Southern, Eastern, and Western Divisions of the Association are carrying on splendid programs in their respective regions. One of the public relations jobs within the Association that we must continually work hard at is to see that these activities are properly coordinated towards achieving our major objectives. I extend to the officers, committee members, and representatives, particularly, and to all others who have assisted them in one way or another, my appreciation for their cooperation and good work during the year.

At this time I can comment on only a few of the activities in which we are engaged. The Executive Board and officers this year have been especially concerned with (1) the business organization, which has been studied by a special committee under the leadership of the venerable Dr. Walter Price; (2) modernization of the Constitution and By-laws under the leadership of Vice-President Dr. E. L. Jack; (3) developing greater international relations, (since 10% of the membership and 60% of the subscribers reside in foreign countries), studied by a special committee under the leadership of Dr. I. A. Gould; (4) public relations and a study of what the membership composition of the Association should be, by the Policy Committee; (5) study of rules and procedures for Association Awards by a special committee under the leadership of Dr. G. W. Salisbury, and (6) the development of an outstanding dairy manufacturing extension program under the leadership of Professor R. B. Redfern. You will be apprised of the results of these and other activities during the course of the meeting.

My deep and sincere thanks go to our wonderful Secretary, Dr. H. F. Judkins. This man must have had his start in the dairy industry, because he surely knows how to work. At personal sacrifice, he has devoted his energies to the work of this Association. No detail escapes him, no job is too menial for his attention, no task is too great for him to tackle. A wealth of information about the Association is at his command, he is ever working and prodding officers and committee members to greater effort. So Dr. Judkins is our fortune and I am sure you all wish to join me in thanks for his distinguished presence among us.

As you know, last year a National Student Affiliate Branch was established by the Association. This is a reflection of the deep interest we have in the student who is planning a career in the dairy industry. This, together with the Student Chapter and the student paper presentation award program, is important to the future of this Association. It is also important from the standpoint of recruitment of young people for careers in the dairy industry. A good number of student affiliate members are present at this meeting and they have an excellent program that deserves the attention of our adult members. Dr. J. E. Johnston and his co-workers have done an excellent job in organizing this new Section and its program.

For the past several pears the Executive Board has sought to increase the interest in education, subject matter teaching, and dairy curriculums in the program of our annual meeting. The Education Committee, under the leadership of Dr. B. L. Herrington, has organized an excellent program of speakers that will effectively focus attention on this subject. As time passes, I hope that this Committee will greatly widen its scope of activity on this important subject.

The activities of the Public Health Committee are especially important at this time. The problem of possible harmful contamination of the food supply by chemicals that might be used in production has not escaped milk and its products. There is much need for accurate public information, the pointing out of research needs, and extension education of procedures in the wise use of these materials in production. The Public Health Committee is functioning in this area. The lecture on Thursday morning before the general session by Dr. H. E. O. Heinemann dealing with pesticide residues points up the importance of this problem and will be of interest to all of you.

Through the years the JOURNAL OF DAIRY SCIENCE has maintained a constant high standard among technical journals. The job of producing this journal is tremendous. The Journal Management Committee, the Editorial Board, and especially the Editor, Dr. E. O. Herreid, have our special thanks for their outstanding contributions. The Publisher, the Garrard Press, has been most cooperative in the issuance of the JOURNAL and deserves our thanks. Special appreciation is due those associations and companies that have used our publication for advertising.

In addition to the large number of technical articles included in each issue of the JOURNAL, the Committee and the Editor have developed and included a number of other items more

popular in nature, that have been designed to broaden the reader's interest. They have done a good job with this, but for these features to be most interesting the management deserves greater and wider assistance from the membership.

A year or so ago there was introduced as a part of the JOURNAL a section called Interpretive Summaries. These are popular summaries of each technical article that appears in the JOURNAL. Each author was asked to prepare them, with editing by the Editor. The purpose was to interpret in lay terms for the many nontechnical readers of our publications the findings reported in the technical articles. This has met with varying success. The Journal Management Committee has been studying this problem and last year recommended to the Executive Board that the Interpretive Summaries be published separately as a quarterly supplement to the JOURNAL. The Executive Board, after much debate, since there was divided opinion on the question, accepted the Committee's recommendation to publish a quarterly supplement containing these interpretive summaries. After a period of about 18 months, a study will be made as to the acceptance by the membership of this publication, after which its future will be determined. The first issue of this supplement, known as the ADSA Review, appeared early this summer. I urge each of you who publish articles in the JOURNAL to do your best to cooperate with the Editor and Journal Management Committee in preparing popularized informative summaries of your research for this ADSA Review. I believe that if this project is successful it may lead to what many of us have longed for, a truly popular dairy science journal of the caliber of the JOURNAL OF DAIRY SCIENCE.

The Association over the years has developed a fine awards program, well supported by the dairy industry. This program gives proper recognition to individuals for outstanding achievement. This is essential in the development of talents and in attracting scientists and educators to our fold.

Just a year ago I assumed the office of President of this fine Association. It has been a rewarding experience. I have tried to be mindful of the responsibility that accompanied this privilege of being your President. At the conclusion of this meeting, Dr. E. L. Jack of the University of California will become your new President. He is well trained for the job and is most able. I know that he can look forward to the same loyal, effective support that each of you has given to me.

THE AWARDS PROGRAM

The Awards Program took place on the evening of June 13. Professor George Werner, Extension Dairyman at the University of Wisconsin, was master of ceremonies and he introduced The Six Foot Four barbershop quartet, who entertained with several humorous selections.

John A. Schlegel of Purdue University won the Graduate Student Scientific Paper Award in Manufacturing and was presented with a check for $100 and an appropriate certificate

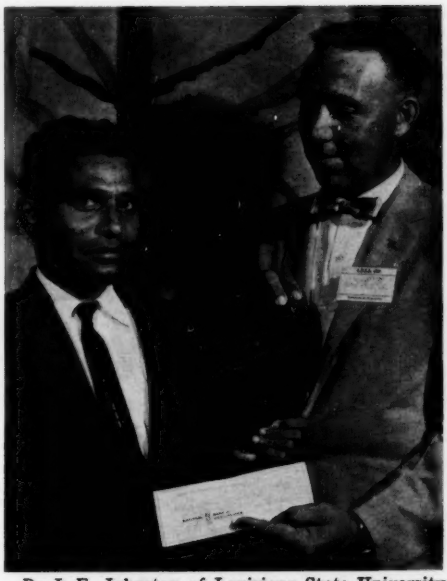
America's Dairy Princess, Bonnie Sue Houghtaling of Swan, Iowa, arriving for the Annual Meeting of the American Dairy Science Association and being greeted by Wisconsin's Alice in Dairyland, Joan Engh (left) of LaCrosse, Wisconsin, and Lewis D. Meyer, President of the Student Branch of the Association, from Michigan State University.

Dr. W. F. Shipe of Cornell University presenting the Graduate Student Scientific Paper Award in Dairy Manufacturing to John A. Schlegel of Purdue University.

Dr. Louis Boyd of the University of Tennessee presenting the Graduate Student Scientific Paper Award in Dairy Production to J. S. Sutton of Iowa State University.

from the Association, by Dr. W. F. Shipe of Cornell University. J. D. Sutton, Iowa State University, won the Graduate Student Scientific Paper Award in Production and was presented with a check for $100 and a certificate from the Association, by Professor Louis Boyd, University of Tennessee.

The Dairy Science Club of the University of Georgia was judged as having the best exhibit of its activities and was awarded a check for $100 and a plaque from the Association, by Professor J. E. Johnston, Louisiana State University, Chairman of the Student Affiliate Committee.



Dr J. E. Johnston of Louisiana State University presenting the Affiliate Chapter Award to K. S. Hedge, who represented the Dairy Science Club at the University of Georgia for having the best exhibit.

National officers of the student branch of the American Dairy Science Association. Jacob Megehee, Mississippi State University, first Vice-President; C. Christman, Michigan State University, Secretary-Treasurer; Joe Lineweaver, Virginia Polytechnic Institute, President; Keith Jenkins, Ohio State University, second Vice-President; Dennis Boesiger, University of Nebraska, third Vice-President; Professor J. B. Mickle, Oklahoma State University, adviser; and Professor W. F. Shipe, Cornell University, adviser.

Student affiliates who attended the annual meeting of the American Dairy Science Association at the University of Wisconsin, 1961. Front row: Francis Rothange, Secretary-Treasurer, 1960-61, Oregon; Lewis Meyer, President, 1960-61, Michigan; Dennis Boesiger, 3rd Vice-President, 1961-62, Nebraska; Keith Jenkins, 2nd Vice President, 1961-62, Michigan; Camilla Christman, Secretary-Treasurer, 1961-62, Michigan; Jacob Megehee, 1st Vice-President, 1961-62, Mississippi; Joseph Lineweaver, President, 1961-62.

Second row: Arthur Smallreed, Ohio; John Murray, Ohio; Wayne Hill, South Dakota; Morey Miller, Pennsylvania; Roy Smith, Colorado; Robert Rasser, Oklahoma; Stanley Gilliland, Oklahoma; Frederick Bowers, Michigan; Fred Ahalt, Virginia.

Third row: Tom Cooper, Kentucky; Warren Suchovsky, Michigan; Coromuto Perez (Venezuela), Michigan; Paul Tassie, Montana; George Shook, Pennsylvania; James Keith, Virginia; David Macomber, Illinois; Herbert Streaker, Maryland; John Healy, Minnesota.

Fourth row: Zane Akins, Missouri; Robert Smariga, Maryland; Nathan Zimmerman, Michigan; Marvin Oetting, 3rd Vice-President, 1960-61, Missouri; Robert Van Pelt, 2nd Vice-President, 1960-61, Washington; George Martin, Tennessee; James Seibel, Wisconsin; Edmund Heinle, Montana; Robert Goodwin, Virginia; Hiel Hicks, Michigan.

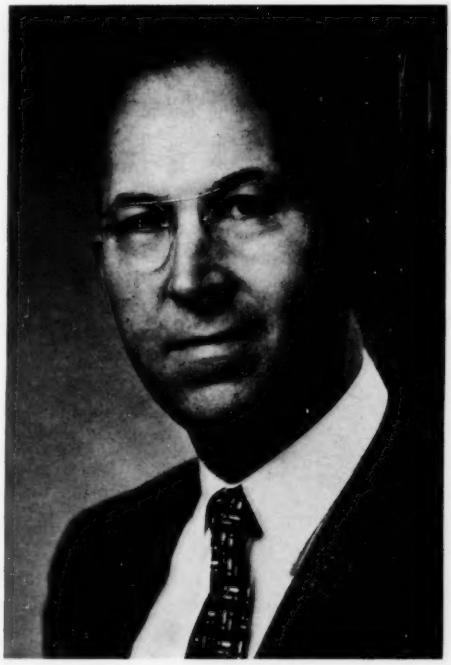
Fifth row: Ernest Girorroid, Louisiana; Dr. J B. Mickle, Advisor, Oklahoma; Dr. J. B. Williams, Advisor, Minnesota; Ken Walker, Colorado; Dr. J. E. Johnston, Student Affiliate Committee Chairman, Louisiana; R. H. Klieber, Oregon.

Citation by Dr. Bruce Poulton for the Recipient of the Milk Industry Teaching Award in Dairy Manufactures for 1961

This year, our American Dairy Science Association takes particular pleasure in recognizing a native son of Wisconsin, our great dairy state, for his contribution to the teaching of dairy science. This year's recipient was born and reared in Wisconsin and soon recognized that Wisconsin was truly America's dairyland, and has, therefore, devoted some forty years of his life to the teaching of dairy science in this great state. Remarkably, he has found time during these years to also author more than forty articles and papers which have contributed greatly to our knowledge of dairy science. His keen interest in teaching has resulted in the preparation of four teaching manuals in the dairy science field. Truly, he has lived The Wisconsin Idea, that the boundaries of the State are the boundaries of the University. His counsel has been sought and freely given everywhere in Wisconsin's dairy industry.

Mr. Hubert Garrecht presenting the Milk Industry Foundation Dairy Science Teaching Award to Professor L. C. Thomsen, with Dr. Bruce Poulton (right), who read the citation.

Our recipient, who is a gifted and devoted teacher, is an alumnus of the University of Wisconsin and began his career at this University following his service in World War I.



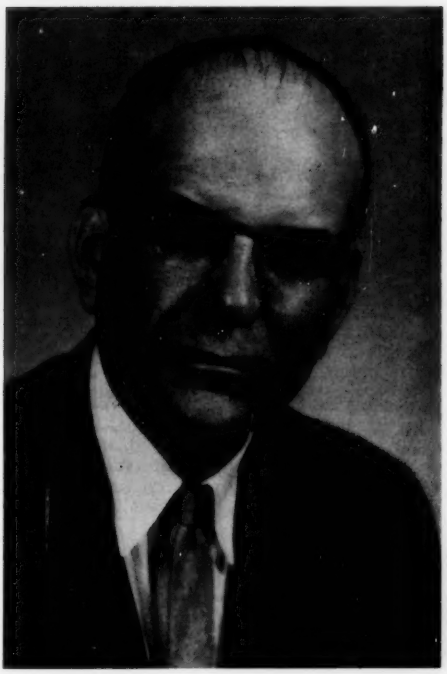
L. C. Thomsen

He started as a teaching assistant in 1919, then climbed the ladder of success and recognition, finally reaching the distinguished rank of Professor of Dairy and Food Industry, which he has held for more than 16 years. In the forty years he has actively advised students, he has guided and stimulated hundreds of students, many of whom have distinguished themselves not only in the dairy industry but in other pursuits as well. Included in this distinguished group there was a young man named Conrad Elvehjem, who now guides this great University. Through the years he has given generously of his time in the development of our American Dairy Science Association. He has served on many of our committees. In recognition of all this, it is my distinct pleasure to call to the platform for our deserved acclaim, Professor Louis Charles Thomsen of the University of Wisconsin.

Citation by Professor Raymond Albrectsen for the Recipient of the DeLaval Extension Award for 1961

The candidate for the 1961 De Laval Extension Dairyman Award was born in Ohio just a few years after the turn of the century. His earliest honors, to the best of our knowledge, were winning the State Corn-Growing Contest for High School students for two consecutive years.

He graduated from Ohio State in 1926 with a B.S. degree and earned his Ph.D. degree in 1936 at American University Graduate School in Washington. In 1933 he was given the special assignment by the United States Department of Agriculture of investigating the dairy herd improvement programs (DHIA) in the leading dairy countries. This investigation provided the basic data for the establishment of the present nation-wide production testing and sire-proving programs.



J. F. Kendrick

The candidate has worked closely with Extension dairymen in the several states in bringing about a standardization of production testing and sire-proving procedures on a national basis, so that a more effective educational program of herd improvement could be carried on with cooperating dairymen. He developed age-correction factors for all dairy breeds for use in sire proving. Studies on the data needed for significant sire proofs were carried on constantly. He developed many procedures for increasing the significance and usefulness of DHIA data. He developed a national system for identification of animals under production test and those in health control programs, and a national system of identification of herds in

DHIA programs. He provided the leadership in furnishing the dairy industry with one of the most comprehensive sire evaluation systems ever developed.

He early recognized the tremendous source of research data, in the accumulation of millions of lactation records. He also recognized the need for a DHIA record that had greater application and usefulness as a teaching aid in assisting dairymen to adjust to changing economic and production situations. He led in the use of electronic data processing equipment and gave much encouragement and technical advice in the establishment of the several regional dairy records processing laboratories.

Mr. H. B. Bradshaw, of the DeLaval Separator Company, presenting the DeLaval Extension Award to Dr. J. F. Kendrick, with Professor Raymond Albrectsen, who read the Citation.

The candidate has been honored twice by the Secretary of Agriculture for Superior Service. He served as Chairman of the Extension Section of the American Science Association in 1942. He served as Chairman of the Sire Committee and Dairy Records Committee of the Extension Section. Currently, he is an ex-officio member of the Dairy Records Committee and Chairman of a sub-committee on Electronic Data Processing of DHIA records.

To these many honors and recognitions of outstanding service to dairymen and to his associates in the dairy industry is now added the De Laval Extension Dairyman Award. On behalf of the committee, it is a pleasure to ask Dr. J. F. Kendrick to come to the platform to receive a well-earned honor.

Citation by Dr. E. E. Bartley for the Recipient of the American Feed Manufacturers Award for 1961

The recipient of the American Feed Manufacturers' Award for 1961 was born in Chicago, Illinois, in 1928. He received the Bachelor of Science degree in 1950 from Colorado State University and the Master of Science degree in 1951 from the same institution. In 1955, he earned his Ph.D. degree at Michigan State University.

Since receiving his Ph.D. degree he has been engaged in dairy nutrition teaching and research, with emphasis on studies dealing with rumen function, digestion, microbiology, and physiology. During the past three years the recipient has authored or co-authored 17 re-

R. S. Emery

search papers in the field of dairy cattle nutrition. His research work has been characterized by originality, thoroughness, depth, usefulness, and represents several approaches to obtaining fundamental information on dairy cattle nutrition. Most of his publications present information beyond that obtained from a common feeding trial. Much of this basic information has already found practical application in various phases of dairy production.

The recipient's research work has dealt extensively with the rumen phenomenon of bloat, its causes, prevention, and control. Other useful research contributions include measurements of the efficiency of rumen function, the oxidation-reduction potential of the rumen, effect of ethanol on rumen fermentation, and saliva production and its importance in rumen function. Notable, too, is his work pertaining to urea utilization by ruminants and how it

Dr. J. Wesley Nelson presenting the American Feed Manufacturers Association Award to Dr. R S. Emery, with Dr. E. E. Bartley, who read the Citation.

can be increased, and effects of rumen factors such as valeric acids, sulfite waste liquor, and calcium gluconate on growth, milk production, and metabolism.

Since receiving his Ph.D. degree in 1955, the 1961 Award winner has been a staff member of Michigan State University.

On behalf of the selection committee and the Association, it is my pleasure to ask Dr. Roy S. Emery to come forward to receive the 1961 American Feed Manufacturers' Award.

Citation by Dr. W. J. Harper for the Recipient of the Borden Award in Dairy Manufactures for 1961

This year's recipient of the Borden Award in Dairy Manufactures began life a half-century ago in St. Paul, Minnesota. His early education was obtained in South Dakota, where he received his Bachelor's degree in Chemistry from Augustana College in 1936. He taught mathematics for a year, then headed high school science programs for the next three years in both North Dakota and Illinois. Entering the University of Illinois in 1940, he majored in physical chemistry and received his Ph.D. degree in 1944. He entered the Dairy Industry as a Research Chemist for the Dean Milk Company in Rockford, Illinois, for 2 years, then returned to the field of education.

During the past 14 years he has engaged in both teaching and research in Dairy Chemistry. Perseverance, patience, and a cheerful willingness to assist others are noteworthy attributes of our recipient. His principal research has been in the area of fundamentals, which has provided no quick answers but has laid a firm foundation towards the ultimate understanding of the complexities of the milk system. He has conducted research in such diverse fields as flavor chemistry, protein chemistry, ultrasonics, analytical chemistry, and biochemistry. Among

R. M. Whitney

his major contributions are these relating to the characterization of components of the stale flavor of dried milk; characterization of milk proteins, with emphasis on their native unaltered state in milk; development of an ideal buffer system for physical chemical studies of milk proteins; improvement of methods for studying the heat stability of milk proteins; and development of improved analytical methods for nitrogen, citrate, and basic cations in

Mr. J. H. McCain of the Borden Company Foundation presenting the Borden Award in Dairy Manufacturing to Dr. R. M. Whitney, with Dr. W. J. Harper, who read the citation.

milk and the investigation of the fundamentals of ultrasonic destruction of microorganisms.

Our recipient is an active member of Sigma Xi, Gamma Sigma Delta, the American Dairy Science Association, and the American Chemical Society.

Our sincere congratulations are extended to our recipient from the University of Illinois—Dr. Robert McL. Whitney.

Citation by Dr. H. A. Herman for the Recipient of the Borden Award in Dairy Production for 1961

The recipient of the Borden Award in Dairy Production for 1961 was born May 28, 1917, at Jerseyville, Illinois. He graduated from the Jersey Township High School in 1935 and was active in 4-H Clubs and in the Future Farmers of America.

He received his B.S. degree in Agriculture in 1940 from the University of Illinois and his Master's degree from Purdue University in 1942. From May 1943 to 1946 he served in the United States Navy in the Pacific operations and was discharged as Lieutenant (J.G.). He returned to Purdue and was awarded his Ph.D. degree in February, 1947.

Except for service in the Navy, our recipient has been most active in teaching and research in dairy production since 1940. His research has covered various phases of animal reproduction, cattle nutrition, composition of milk, calf raising, endocrinology, and animal physiology.

R. E. Erb

His research has involved numerous studies of semen quality and fertilizing capacity; he has published numerous papers on fertility as influenced by the female; he has analyzed the effects of estrus and gestation on milk production; his studies involve a most thorough analysis of the breeding picture in a well known herd where accurate records are available of the past forty years; he has also demonstrated the value of monthly, bi-monthly, and tri-monthly tests in measuring milk production. In recent years, he has concerned himself with measures of nonfat solids in milk and practical measures under field conditions. A rough check indicates more than 40 papers in the JOURNAL OF DAIRY SCIENCE attributed to him and fellow workers. He also has many experiment station bulletins, circulars, and popular papers to his credit.

Our candidate has held several important offices in A.D.S.A. including: Chairman, Breeds Relations Committee; Chairman, Production Section and Secretary-Treasurer of the Western Section of A.D.S.A. He is past Chairman of the Northwest Regional Breeding Project, Cause and Prevention of Breeding Failures in Dairy Cattle. He also serves as a dairy judge at many shows.

His academic records include several years at Purdue University as Instructor in Dairying. In 1947 he moved to Washington State University, Pullman, Washington, as Assistant Professor and in 1953 was appointed Professor of Dairying.

The extensive and active participation of this man for the past 21 years in key dairy production research is known to all, and most of you have read reports of his work.

It is a sincere pleasure on the part of the 1961 Committee for The Borden Award in Dairy Production to ask Dr. Ralph E. Erb, Washington State University, to come forward.

Mr. J. H. McCain of the Borden Company Foundation presenting the Borden Award in Dairy Production to Dr. R. E. Erb, with Dr. H. A. Herman, who read the Citation.

Citation by Dr. M. E. Powell for the Recipient of the Paul-Lewis Award in Cheese Research for 1961

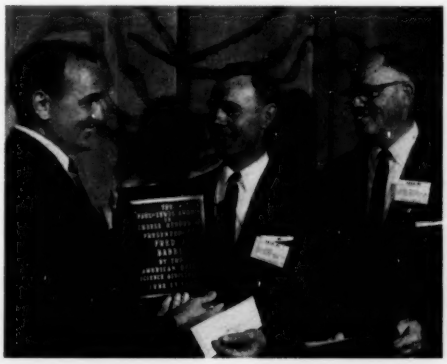
The person chosen by the committee to receive the 1961 Paul-Lewis award in cheese research was born in Traverse City, Michigan. He received a B.S. degree from Michigan State College in 1935. That same year he was awarded a one-year fellowship at Purdue University by the Dairy Industries Supply Association. This led to an M.S. degree from Purdue in 1936. Continuing his graduate studies at Iowa State College, he earned the Ph.D. degree in 1939, and continued on the staff there until 1947. Since that time he has been at Purdue University.

The recipient has been actively engaged in research connected with cheese for more than 15 years. He has dealt largely with bacterial cultures, so essential for cheese production. His studies have been concerned with the flavor characteristics and activity of the cultures. He is an authority on bacteriophages which are active against lactic streptococci, and he has worked diligently to discover means for controlling the virus. The work has involved the use of phage-resistant media in propagating starter cultures. He has been active in the development and selection of strains of organisms resistant to various phages and the use of these resistant strains in cheese production.

A significant contribution to the cottage cheese industry was his method for increasing and standardizing the level of flavor through the use of carefully selected cultures incorporated in the cream dressing. Not only is the flavor enhanced, but the keeping quality of the cheese is materially increased because the cultures have definite inhibitory characteristics toward common cheese spoilage organisms. He was awarded a patent for this novel application.

In addition to his numerous original articles, he has published several lengthy reviews dealing with lactic cultures. He is co-author of one of the prominent textbooks on Dairy Bacteriology.

F. J. Babel



Mr. C. B. Dedrich of the Paul-Lewis Laboratories presenting the Paul-Lewis Award in Cheese Research to Dr. F. J. Babel, with Dr. M. E. Powell, Knudsen Creamery Company, Los Angeles, who read the Citation.

Our recipient has been active in the affairs of the American Dairy Science Association. He has served in all offices of the Manufacturing Section, and was General Program Chairman for the annual meeting in 1959.

On behalf of the Paul-Lewis Award Selection Committee, it is a distinct pleasure to present the recipient of the 1961 Award, Dr. Frederick J. Babel, Professor of Dairy Bacteriology, Purdue University.

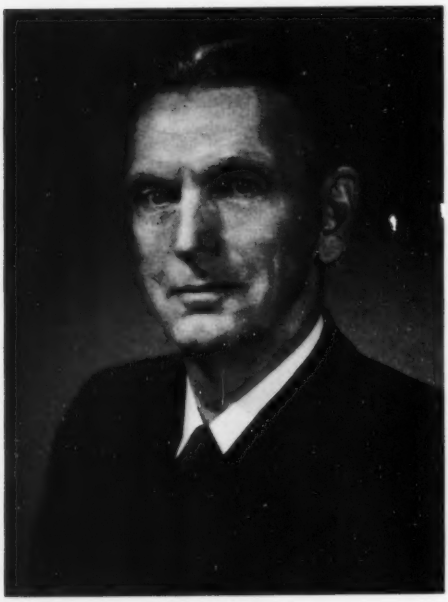
Citation by Dr. N. P. Ralston for the Recipient of the American Dairy Science Association Distinguished Service Award for 1961

One of the two candidates to receive the 1961 Distinguished Service Award was born in Merrill, Michigan. He attended Oberlin College for one year and then earned his Bachelor's degree in Literature in the School of Journalism, Columbia University, in 1915.

Upon graduation he was employed by the

New York *Tribune*, the New York *World*, and *Air Travel*—a magazine. He was the publisher and editor of the latter in 1917. He served his country for two years during the First World War.

Since this time he has devoted his professional life to the application of fundamental principles of organizational administration and management for the welfare of the dairy industry. As our industry has grown and flowered into an accepted and mature business, our candidate has brought about coordination and stability within the dairy manufacturing industry. He has used his literary and journalistic talents to continually foster sound public relations concepts to aid in the creation of a favorable image of our industry, both within the United States of America and internationally.



Roberts Everett

This man possesses and has demonstrated genuine qualities of dairy industrial leadership. He has been able to encourage the utilization of resources for the creation and synthesis of ideas that have united our industry for important mutual concerns. The very nature and requirements of his past work have demanded that he be a keen student of the behavior of businesses and businessmen, thus necessitating the use of great skills in the management process, both formally and informally.

The recipient of this award was the first employee of the Dairy Industry Supply Association in 1919. When he retired as Executive Vice-President in 1960, it had 424 members and an annual budget of over a quarter million dollars. He initiated the Dairy Industries Exposition. He was an originator and vigorous promoter of the 3-A Standards for Dairy Equipment. Since 1930 he has enthusiastically and firmly supported the Collegiate Students International Contest in Judging Dairy Products. The development of the Dairy Society International is one of his lasting dairy industry developments and humanitarian contributions to the people of the underdeveloped countries of the world. Under his leadership and guidance through this latter organization, the First World Congress on Milk Utilization was held in 1953.

Dr. R. E. Hodgson (right) presented the American Dairy Science Association Distinguished Service Award to Roberts Everett, with Dr. N. P. Ralston, who read the Citation.

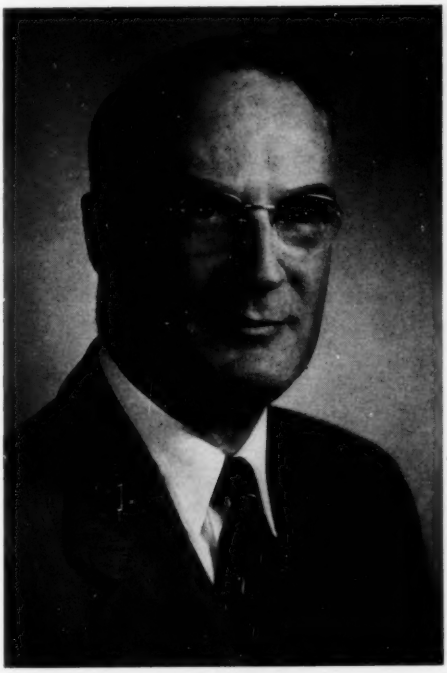
Because of his truly vigorous, aggressive, and sound leadership in organizations of our dairy industry, and many other closely allied industrial and business activities, the Committee recommends that the American Dairy Science Association salute this leader by awarding the Distinguished Service Award to Mr. Roberts Everett, Supervisory Consultant, Dairy Industries Supply Association, Washington, D. C.

Citation by Dr. N. P. Ralston for the Recipient of the American Dairy Science Association Distinguished Service Award for 1961

One of the two recipients for the 1961 Distinguished Service Award is a native of the Badger State. He received both his Baccalaureate and Master's degrees in dairy manufacturing at the University of Minnesota in 1915 and 1916, respectively. Thirteen years later, in 1929, he earned his doctorate at the University of Illinois.

Our recipient held a graduate fellowship at the University of Minnesota from 1915 to 1916, followed by an instructorship in dairy manu-

factures at the University of Wisconsin, 1916 to 1918. He was a creamery extension specialist at North Dakota Agricultural College for one year and superintendent of a cooperative creamery at Fargo, North Dakota, for two years. For twenty-two years he has been Chief in Research at one of our Agricultural Experiment Stations and has been a Professor of Dairy Industry since 1941 at one of our leading universities.



A. C. Dahlberg

The candidate for this meritorious award belongs to many societies, among which are the honor societies of Sigma Xi and Gamma Sigma Delta. He served our American Dairy Science Association as a director, vice-president, and president. He was editor of our JOURNAL for ten years. In addition, he has served on many committees of our Association. He has functioned effectively and efficiently in significant positions in state, federal, and international organizations and activities in the interest of furthering the American dairy industry and building dairying as a profession. He received the Borden Award for Distinguished Research in Dairy Manufactures in 1944.

The man to receive this citation this evening for distinguished service to the dairy industry is currently holding four most important consulting positions with industry and federal

Dr. R. E. Hodgson (right) presented the American Dairy Science Association Distinguished Service Award to Dr. A. C. Dahlberg, with Dr. N. P. Ralston, who read the Citation.

agencies. Our recipient has published numerous scientific research and popular articles in scientific journals, popular magazines, bulletins, and special reports dealing with milk, cream, ice cream, cheese, and some phases of dairy herd management.

It is with real pride and sincerity that the Distinguished Service Award Committee recommends to you Dr. A. C. Dahlberg, Professor of Dairy Industry, at Cornell University, to receive a 1961 American Dairy Science Distinguished Service Award.

Citation by Dr. D. V. Josephson for the Recipient of the American Dairy Science Association's Award of Honor for 1961

The highest recognition that the American Dairy Science Association can extend to any of its members is its Award of Honor. This year it is our privilege to recognize and honor a distinguished dairy scientist and teacher, a man who is now concluding a 39-year career in the field of dairy cattle nutrition research and education.

He was born and reared on a farm near Tonganoxie, Kansas. After finishing high school he entered Kansas State College and completed his B.S. degree in 1917. World War I temporarily interrupted his further education, but after serving in the United States Air Force as a flight instructor for the duration of the war, he returned to pursue graduate studies at the University of Minnesota, where he received his M.S. degree in 1922. He immediately accepted a position at the then Michigan Agricultural College (now Michigan State University) where, with the exception of one year when he took leave of absence to complete his Ph.D. at the University of Wisconsin,

C. F. Huffman

he has devoted his entire professional life to research and teaching.

During his career at Michigan State he has held positions of research assistant, research associate, associate professor and, since 1938, the rank of research professor.

His long and distinguished service to his University and to the dairy industry is widely known. The variety of his sustained interests during these nearly four decades of dairy research precludes even a superficial listing here. Perhaps a reasonable share of his research activities can be classified under three areas, mineral metabolism, evaluation of feedstuffs, and rumen physiology. His work, primarily, is the basis for our present phosphorus requirements for milk production. His extensive studies of minerals have also included the effect of magnesium deficiency and its relationship to vitamin D requirements, calcium-phosphorus relationships, cobalt metabolism, and the effects of fluorine. A keen observer, as well as a trained scientist, his insight into the complexities of bovine digestion has contributed much to our knowledge of the nutritive value of feeds. He stressed the importance of a balanced ration and has made major contributions to our knowledge of nutrient synthesis in the rumen, using special fistula techniques.

He has always had an enthusiastic group of graduate students around him, of which more than 20 have completed their Ph.D. degrees under his direction. He has authored more than 125 papers published in scientific journals, particularly in our JOURNAL OF DAIRY SCIENCE. He has the unique ability of translating highly scientific reports into accounts which are understood and enjoyed by the layman. His use of titles such as, Why a cow's breath is sweet, The inside story of the cow, and The romance of the gut, demonstrates his flair for popularizing science to the layman.

Dr. R. E. Hodgson (right) presented the American Dairy Science Association's Award of Honor to Dr. C. F. Huffman, with Dr. D. V. Josephson, who read the Citation.

This man has served our Association extensively and well. Throughout his professional career he has participated on innumerable committees in Association affairs and climaxed his service as Vice-President and President in 1956 and 1957, respectively. In all of his assignments and offices he has worked with efficiency and enthusiasm.

He has been the recipient of many honors. In 1937 he was the first recipient of the Borden Award in Dairy Production. Again, in 1954, he was honored as recipient of the American Feed Manufacturers Award. In 1946, the Michigan State Chapter of Sigma Xi presented him its Senior Research Award and in 1960 Michigan State University selected him for the Distinguished Faculty Award.

Now we, the American Dairy Science Association, are privileged to honor this distinguished gentleman. While our Award of Honor does not carry with it the $1,000 to which he has become accustomed, it does include over 2,500 salutes and best wishes from the members of this Association. Tangible evidence of this recognition will be found on this seroll. The American Dairy Science Association, this year, bestows its highest recognition—The Award of Honor—on Dr. Carl F. (Shorty) Huffman of Michigan State University.

INSTALLATION OF OFFICERS

President R. E. Hodgson installed the officers immediately after the Awards Program. Raymond Albrectsen and C. W. Nibler were installed as Directors; I. W. Rupel as Vice-President; and E. L. Jack as President.

President Hodgson presented the gavel to President-Elect Jack, who pinned the Past-President's badge on Dr. Hodgson, and the meeting was adjourned.

THE PAST-PRESIDENTS DINNER

The annual Past-Presidents dinner was held on June 12 in the Memorial Union Building, where 16 past executives and the Board of Directors enjoyed an excellent roast beef dinner.

Before the dinner the Past-Presidents participated in a discussion of the subject, How Can the Annual Meeting of the A.D.S.A. Be Improved?

Dr. A. C. Dahlberg of Cornell University was the first speaker and stated that a longer and less crowded meeting schedule is needed. He suggested that the Executive Board might start its meeting a day earlier than at present and have at least a half day before the start of the Annual Meeting, allowing more time for visiting university facilities and making tours. Dr. Dahlberg agreed that it would be difficult to decrease the number of papers submitted and recommended that an extra day for the Annual Meeting be considered, although he felt that four days would be long.

Dr. W. V. Price of the University of Wisconsin discussed the question of how more members could be brought into Association affairs. He stated that participation was the key to interest in the Association. About 10% of the members do participate as officers and members of various committees. He stated that if matters of policy could be discussed at the business meeting it might enlist greater interest among the members. The formation of an elected council of representatives similar to that of the American Chemical Society was suggested as a means of encouraging participation of members in Association affairs.

Dr. D. V. Josephson of Pennsylvania State University handled the question of how busi-

New officers of the American Dairy Science Association for 1961-62. Seated, Dr. R. E. Hodgson, outgoing President. Dr. E. L. Jack, President; Professor Raymond Albrectsen, Director; Professor C. W. Nibler, Director; and Dr. I. W. Rupel, Vice-President.

American Dairy Science Association's Past-Presidents Dinner. Seated: Earl Weaver, Michigan State University; A. C. Ragsdale, University of Missouri; C. F. Huffman, Michigan State University; J. A. Nelson, Montana State College; A. C. Dahlberg, Cornell University; J. B. Fitch, University of Minnesota; H. P. Davis, University of Neraska. Standing: G. M. Trout, Michigan State University; I. A. Gould, The Ohio State University; H F. Judkins, White Plains, New York; D. V. Josephson, Pennsylvania State University; W. V. Price, University of Wisconsin; W. E. Petersen, University of Minnesota; K. L. Turk, Cornell University; R. B. Becker, University of Florida.

ness affairs of A.D.S.A. can be made more meaningful to the members. He pointed out that the members have a wide variety of interests and that enthusiasm for the business of the Association was especially lacking among teachers and scientists and that industry members feel that the university members are running the Association anyway. When Association affairs are going smoothly, as they are now, there are no reasons for anyone to worry. Dr. Josephson suggested that a series of reports on the business affairs of the Association might well be published in the JOURNAL from time to time. He thought a breakdown by percentage, showing where each type of income comes from and how it is spent, would be of interest. It was suggested that a committee on advertising might be of help to work with the Secretary. It was concluded that we can not expect all members to be interested, but that a more informed membership was needed.

In the general discussion, Dr. John A. Nelson of Montana suggested that the Executive Board might meet at some other time during the year. Vice-President E. L. Jack stated that this was the plan for 1961-62. Dr. D. V. Josephson stated that informal discussions were worth a great deal and more time was needed for them. Dr. W. E. Petersen of Minnesota observed that he had seen many small conferences at this meeting and he thought it would be a good idea to have rooms or offices listed in the program where such impromptu conferences could be held. Professor H. P. Davis of Nebraska thought that releases of abstracts of papers presented should have wide distribution and not be confined to members of the press coming to the press room for them. The Secretary stated that this was being done quite extensively by agricultural editors in the states. Dr. I. A. Gould of Ohio was very enthusiastic in stating that 9 A.M. to 3 or 3:30 P.M. was long enough for scheduled meetings. Dr. Petersen believes it is going to be difficult to interest more members in the business meeting and that Dr. Price's suggestion of an elected council may be a good idea.

GENERAL BUSINESS MEETING OF THE ASSOCIATION

The business meeting was called for the morning of June 14 in the Agricultural Hall Auditorium, immediately after Dr. H. E. O. Heinemann had addressed the joint meeting of all sections on pesticide problems. Unfortunately, many members departed. There were only about 100 persons present at the beginning of the business meeting at 11:30 A.M. and only about 30 people were present when the meeting adjourned at 12:45 P.M. A more appropriate time for the business meeting must be considered in the future.

The reports of the principal committees were summarized by Vice-President E. L. Jack.

Secretary-Treasurer H. F. Judkins presented his report, which appears in the Executive Board minutes. He also discussed some of the

Dr. G. W. Trimberger (right), Cornell University, and Chairman of the Program Committee for the Annual Meeting, looks over the program with Dr. H. E. O. Heinemann (center) and Dr. J. F. Casida, both of whom spoke on pesticide residues in milk.

more important actions that had been approved by the Executive Board.

Editor-in-Chief E. O. Herreid presented a brief summary of his report, which will be published in the JOURNAL.

Dr. H. A. Herman spoke brefly in behalf of the Dairy Shrine Club located at Waterloo, Iowa.

The Constitution and By-Laws amendments, which were printed in the JOURNAL OF DAIRY SCIENCE for April, 1961, were approved.

Dr. S. L. Tuckey, Chairman of the Resolutions Committee, presented the following resolutions, which were approved:

WHEREAS, the members of the American Dairy Science Association who attended the 56th Annual Meeting found the arrangements and accommodations most satisfactory and enjoyable, therefore it is

1. Resolved, that the American Dairy Science Association express its appreciation to the staff of the University of Wisconsin and particularly to the members of the Departments of Dairy Husbandry and Dairy and Food Industry and their families for their friendly hospitality and for all the contributions they made to making this meeting successful.

WHEREAS, the state of Wisconsin is recognized as an area where the dairy industry is highly developed and where the University of Wisconsin has contributed significantly to its development, therefore it is

2. Resolved, that the American Dairy Science Association recognize the contributions and memory of the many individuals at the University of Wisconsin who through their vision, perseverance, ingenuity, scientific teaching, and leadership made lasting impressions on the dairy industry in the United States. We therefore pause to remember them.

WHEREAS, it is appropriate that outstanding scientific research, excellence in teaching, and public service through extension be recognized, let it be

3. Resolved, that the American Dairy Science Association express its appreciation to The Borden Company, American Feed Manufacturers Association, The DeLaval Separator Company, Milk Industry Foundation, National Dairy Products Corporation, and Paul-Lewis Laboratories, Inc., for their generous awards given annually to members of the Association, for superior achievements in research, teaching, and extension.

WHEREAS, the disease Mastitis is the cause of great economic loss to dairy farmers and as yet no unified program for the control of Mastitis in dairy herds has been developed, let it be

4. Resolved, that the American Dairy Science Association commend the National Mastitis Council for its leadership in establishing a national program on Mastitis, that we urge the federal, state, and local authorities, and the dairy industry to review their activities related to the control of Mastitis and to undertake and help support research and educational programs which will eventually provide effective means of controlling Mastitis in dairy herds. Be it also resolved that a copy of this resolution be sent to the secretary of the newly formed National Mastitis Council.

WHEREAS, the use of insecticide and pesticide chemicals are essential in the economical and efficient production of food materials, including milk, and WHEREAS, their improper application may result in chemical residues appearing as adulterants in milk and other dairy products, and WHEREAS, educational programs are the best means of informing producers of proper procedures and hazards in the use of pesticides and insecticides, therefore it is

5. Resolved, that the American Dairy Science Association commend federal, state, and local authorities, the Agricultural extension service, and various segments of the dairy industry for initiating timely programs of assistance and education in the proper and approved methods of using these chemicals. Let it further be resolved, that certain trade associations such as the National Agricultural Chemical Association, National Manufacturing Chemical Association, and the American Feed Manufacturers Association, along with the above-named agencies, be urged to strengthen and improve their programs of research and education pertaining to this problem. Attention should be directed to the problems faced by the milk producers regarding pesticide residues in feeds which are produced in areas remote from the place of use and impossible to control by the purchaser and user of that feed. It is also resolved, that the Secretary of the American Dairy Science Association be instructed to send copies of this resolution to the Secretary of the Department

of Health, Education, and Welfare, and the Secretaries of National Agricultural Chemical Association and American Feed Manufacturers Association, and Secretary of the U. S. Department of Agriculture.

WHEREAS, the specific role of fats in human nutrition has not been fully elucidated, let it be

6. Resolved, by the American Dairy Science Association, that it commend those agencies of the federal government such as the National Institute of Health, (Dept. of H.E.W.), the Institute of Human Nutrition, (USDA), University and College Agricultural Experiment Stations, the American Dairy Association and the National Dairy Council, and others who are conducting and fostering research on specific phases of nutrition research, especially milk fat and human health, so that accurate information based on controlled scientific research will be available to all. Let it further be resolved, that these above-named agencies be encouraged to expand and increase their research activities regarding the nutritive value of the milk components other than the lipids. Let it further be resolved, that the Secretary of this Association be instructed to send copies of this resolution to the Secretary of the Department of Health, Education, and Welfare and the Secretary of the U. S. Department of Agriculture.

WHEREAS, there is insufficient information at present regarding the specific role of various factors related to milk composition, let it be

7. Resolved, by the American Dairy Science Association, that all dairy industry agencies be encouraged to continue and expand research projects regarding the genetic, physiological, nutritional, and environmental factors which influence milk composition, particularly the nonfat solids.

8. Be it resolved, That the American Dairy Science Association convey to the Commodity Stabilization Service of the U. S. Department of Agriculture its appreciation of the fine cooperation the New Orleans CSS Office has given the Dairy Cattle Research Branch in providing facilities for the automation of the sire-proving procedures, thereby enabling the Branch to produce proved sire summaries and related data of great value to the dairy industry.

Be it further resolved, That a copy of this resolution be sent to the Administrator of the Commodity Stabilization Service, U. S. Department of Agriculture, Washington, D. C.

9. Whereas, efficiency in milk production is important to the dairy farmer, to the industry at large, and to the consumer, therefore it is,

RESOLVED, that the American Dairy Science Association again strongly urge that the U. S. Department of Agriculture give increased support to the Dairy Cattle Research Branch of the USDA to advance the DHIA production testing and sire-proving programs now under way in the United States. Be it also resolved that the Secretary of the American Dairy Science Association be instructed to send a copy of this resolution to the Secretary of the United States Department of Agriculture.

Respectfully submitted,
J. G. CASH
S. T. COULTER
D. H. JACOBSEN
J. E. LEGATES
S. L. TUCKEY, Chairman

MEETINGS OF THE EXECUTIVE BOARD AND ITS ACTIONS

H. F. JUDKINS, Secretary-Treasurer

The subcommittees of the Executive Board met on June 9 to consider the various committee reports which had been prepared. This made it possible for the Executive Board to act more efficiently on Association affairs. There was an unusually large volume of old and new business and committee reports to consider on June 10 and 11 and briefly again on June 14. The Executive Board actions are presented in the summary that follows.

1. Minutes of 1960 Board Meeting. It was voted that the reading of the minutes of the 1960 meeting presented by the Secretary be dispensed with, since they appear in the August, 1960, JOURNAL.

2. Report on interim actions. Voted to confirm the Secretary-Treasurer's report on actions taken by mail since the 1960 meeting.

a. Constitution By-Laws amendments. Changes were approved by the Board and appeared in the April issue of the JOURNAL. Voted that the amended Constitution By-Laws subject to approval by the membership at this meeting be published in the JOURNAL and reprints obtained for the use of new members and other purposes.

b. Election of life members. The following have been elected to lifetime membership, effective January 1, 1961: Joseph B. Parker, Washington, D. C.; C. E. Wylie, Tennessee; E. M. Harmon, North Carolina.

c. Past President plaques. Pursuant to action of the Board at the June, 1960 meeting, Past-President plaques have been secured and distributed. The Secretary has received very appreciative letters from the Past-Presidents upon their receipt of these plaques.

d. Award eligibility of foreign members. Voted that it be the policy of the Association to confine the monetary awards to members residing in the United States and Canada, and that the possibility of recognition for foreign mem-

The Executive Board in action. G. H. Wise, F. J. Doan, J. T. Reid, Edwine Farrel, H. F. Judkins, N. P. Ralston, R. E. Hodgson, E. L. Jack, E. C. Scheidenhelm, S. T. Coulter, and E. O. Herreid.

bers be referred to the International Relations Committee.

e. Job placement desk at annual meeting. The Board voted against this suggestion, feeling that the results would probably not be commensurate with the effort involved.

f. Past-Presidents dinner. Voted that the Past-Presidents dinner should be paid for from Association funds.

g. Executive Board identification. Pursuant to the action of the Board, red ribbons bearing the word director have been secured for identification of Board members.

h. Attendance at Section Meetings by Board members. The Board felt that while it was desirable that one or more members attend the Section Business Meeting, this should not be made mandatory.

i. A.D.S.A. Review. Pursuant to the action of the Board at the 1960 meeting, to the effect that interpretive summaries should be removed from the JOURNAL and published separately as a quarterly, the A.D.S.A. Review has been developed and No. 1 for the first quarter of 1961 has been printed and distributed to the membership.

j. Election results. The following officers were elected for 1961-62; E. L. Jack, President; I. W. Rupel, Vice-President; C. W. Nibler and Raymond Albrectsen, Directors.

k. Resolution acknowledgment. The Secretary reported that various resolutions passed at the 1960 meeting seem to have been appreciated by various government and state officials to whom copies were sent. Appreciative acknowledgments have been received.

l. Definitions of positions in dairy industry for U. S. Dept. of Labor. The Association has been asked to assist in defining positions in the dairy industry for the U. S. Dept. of Labor dictionary. The Secretary reported that he had secured fine cooperation from a number of our members, both in universities and in industry, in the preparation of these definitions. The job is virtually completed.

3. Dairy Science Abstracts for 1962. Voted that the Board indicate to Dr. Mann that we will accept the proposal submitted, which guarantees 500 copies at $7.50 a copy, and that we strive for the additional 200 copies.

4. Acceptance of future award offers. Secretary announced that all donors of our monetary awards have agreed to make them available for 1962. Letters of appreciation have been sent to them and a resolution thanking them for their generosity will be offered at this meeting.

5. Future meeting dates. The following dates have been confirmed: In 1962, the A.D.S.A. will meet in Maryland, June 18-21; 1963, Purdue, June 17-19; 1965, Kentucky, June 14-16; 1966; Oregon, June 20-22. Arrangements have not been completed for the 1964 meeting.

6. Section Chairman reports. The chairman of each section, including the President of the Student Branch, appeared before the Board and presented excellent statements concerning reports to be presented at their various business meetings. Having section chairmen make these reports to the Board is proving to be an excellent plan. The Extension Section has the problem of approving various new types of equipment and identification procedures involved in DHIA record-keeping. After extended discussion, it was the unanimous decision of the Board that the Association should not approve such equipment or procedures. It was voted to rescind a motion passed at the 1960 meeting which stated that "American Dairy Science Association approval of any program, method, test, or procedure shall be contingent on favorable action by the membership at its Annual Meeting upon recommendation by the Executive Board."

7. Election of new life members. The following were elected to life membership effective January 1, 1962: C. R. Gearhart, State College Pennsylvania; Randall Whitaker, Sayville, New York; L. C. Norris, Cornell Uni-

versity; H. A. Bendixen, Washington State College; John H. Nair, Summit, New Jersey; Roberts Everett, Washington, D. C.; James G. Hays, East Lansing, Michigan; Robert C. Thomas, Carmel, California.

8. President representation on program committee. Voted to have the incumbent President instead of the immediate Past-President an ex-officio member of the Program Committee.

9. Dairy product score cards. Voted that A.D.S.A. print the dairy product score cards and sell them to institutions for their use.

10. Association Business Meeting agenda. The format of the agenda for the 1961 meeting as presented by the Secretary was approved, with the exception that it was voted that any future requests from the Dairy Shrine Club for appearance on the program should be referred to the Extension and Production Sections.

11. Dairy Manufacturing Extension Award. Voted that any action toward securing an award for Dairy Manufacturing Extension be held in abeyance for the time being.

12. Testing standards including certification of media. It was voted to refer to the Manufacturing Section, a letter from Dr. L. A. Black relative to a technical committee meeting of the American Standards Association of the International Organization for Standardization to be held in Berlin and a statement concerning the certification of media.

13. Auditor's report. Voted to accept with commendations the report of the Auditing Committee, which showed a balance of $4,942.33 for 1960.

14. Secretary-Treasurer's report. Voted to accept the report and recommend that the situation with respect to subscribers be studied, with the view of returning the handling of subscribers to the Secretary's office if not being done properly by Garrard Press.

Financial Report
(Calendar Year 1960)

Income	$92,735.40
Expense	$87,792.07
Gain for year	$ 4,942.33

The sources of income and the approximate percentage for each item are as follows:

Source	%
Dues—memberships	28
Dues—Student Affiliates	3.2
Subscriptions	27
Advertising	23
Reprints	14
Back Copies	2
Interest on Investments	1.5
Total	98.7

Expenses were divided as follows:

JOURNAL production costs, including reprints, purchase of back copies,	74.5%
Editorial expenses	9.3%

Summarizing, about 84% of the expenses are involved in publishing the JOURNAL.

Salaries and expenses for the Secretary-Treasurer's office amount to 10%. Miscellaneous expenses amount to 6%; the largest item is concerned with officers' travel to committee meetings and to the Annual Meeting.

Total accumulated reserve as of December 31, 1960, is $47,916.44

Thus far, this financial report has been most favorable, but some factors unforeseen last year and appearing this year, may cause a deficit of $12,000–$15,000 which will have to be taken out of our reserves. This is due, partly, to the fact that an increase in the amount of grants available for research has increased the number of papers being received for publication in the JOURNAL and this trend will continue. This seems desirable and our JOURNAL should take care of these papers. For example, in January, 1960, the total number of pages in the JOURNAL was 162, whereas in January, 1961, the number was about 220. It appears our JOURNAL cost is increasing at an average rate of about $1,000 a month. Furthermore, the average cost per issue is approximately $1 per copy, or $12 per year.

Due to austerity programs, some companies are not spending money for advertising this year as they did last year. While efforts to obtain advertising for 1961 have been redoubled, we shall probably have a shrinkage of $3,000–$4,000 in our 1961 advertising.

The Board and officers have developed a budget for the year 1962 of $110,198. They realized our financial situation and have voted the following increases in rates, effective January 1, 1961:

Member Dues—From $10.00–$12.50
Subscriptions—From $15 domestic and $15.50 foreign to $20 and $20.50, respectively.

These new rates, incidentally, will put our Association more in line with the rates being charged by various other scientific organizations.

The Board also voted to advance the price of reprints 25% and to make a charge of $2 per individual copy for back issues, both changes to become effective July 1, 1961.

The Board has voted in favor of $100 a year sustaining memberships, which it is hoped may be obtained by contacting various associations and companies within the industry. Several other organizations follow this plan. This fall, when the dues notices are submitted, you will be asked to vote on an amendment to the Constitution which will establish sustaining memberships.

That there may be no misunderstanding, it may be said that the A.D.S.A. Review, which has been started this year as a result of Board action of 1960, is not responsible for the increase in the cost of JOURNAL production. Several months ago it was determined that the savings derived from omitting the interpretive summaries from the JOURNAL would pay for the cost of the quarterly Review containing these summaries. As a matter of fact, the cost of printing these summaries in the quarterly A.D.S.A. Review is slightly less than printing them in the JOURNAL.[1] The over-all saving has not resulted due to the fact that the space formerly occupied by the summaries in the JOURNAL has been more than taken up by more papers that have been presented for publication.

Our printed Training Manual project shows a small profit and we have about 800 copies on hand.

We still have on hand 500 copies of the Supplement to the April, 1960, JOURNAL, Papers of the 4th Biennial Symposium on Animal Reproduction. This project still shows a small deficit.

MEMBERSHIP

The total number of members in 1960 was 2,579 and affiliate members, 605. As of June 8, 1961, the number of members was 2,543 and affiliate members, 656. Thus, the number of 1961 members may be expected to be about the same as for 1960, whereas we have had a good increase in student affiliate members, undoubtedly largely due to the activities of the new Student Branch. As usual, nearly 300 members in 1960 have not renewed for 1961. The vast majority of these have been members for only 1 or 2 years. Thirty-six per cent of those who have not renewed are engaged in Dairy Manufacturing activities, indicating that they were not satisfied with the Association.

The Necrology report is the saddest part of this report. It has been reported that the following have passed away since the last meeting:

H. C. Avis, Greenbriar Dairy Products Co., Beckley, W. Virginia

E. K. Berg, Central Ohio Cooperative Milk Producers, Inc., Columbus, Ohio

Mason Campbell, University of Rhode Island, Kingston, R. I.

Robert R. Fast, Hermiston Veteran's Hospital, Hermiston, Oregon

J. D. Foster, University of Kentucky, Lexington, Kentucky

H. C. Jackson, University of Wisconsin, Madison, Wisconsin

P. G. McGilliard, Oklahoma State University, Stillwater, Oklahoma

William D. McMillan, Cooperative G.F.I., Exchange, Inc., Ithaca, N. Y.

R. H. Olmstead, Pennsylvania State University, State College, Pa.

Verne C. Summers, Private Laboratory, Lowell, Indiana

Clifford R. Swett, National Wax Company, Skokie, Illinois

J. O. Tretsven, Retired dairy specialist of the Montana Agricultural Extension Service, Bozeman, Montana

R. Washburn, Ohio State Experiment Station, Wooster, Ohio.

Mr. President, will you ask the meeting to stand in a moment of silence out of respect to the men whose names have just been read.

15. Approval of budget for 1962. Voted to adopt the budget as amended, in the amount of $110,198.

16. Editor's report. Voted to accept the Editor's report and that it be edited for publication in the JOURNAL.

17. Selection of Editor. Voted to reappoint Dr. E. O. Herreid as Editor-in-Chief, with a note of appreciation for the outstanding contributions he has made in advancing the standards of publication for the A.D.S.A.

18. Selection of Secretary-Treasurer. Voted to reappoint Dr. H. F. Judkins as Secretary-Treasurer, with a note of appreciation and commendation for excellent, devoted service to the Association.

19. Selection of Journal Management Committee member. Nominees were B. H. Webb, J. R. Brunner, and F. J. Doan. Voted that the Secretary be authorized to cast a unanimous ballot for F. J. Doan.

20. Selection of Policy Committee members. The Board elected K. L. Turk and N. L. Jacobson as new members, with K. L. Turk Chairman of this Committee.

21. Selection of Program Committee Chairman for 1962. Voted that the Board elect Burdet Heinemann as Chairman of the Program Committee for the 1962 meeting. (Note: Unable to serve and Professor Marvin E. Senger of North Carolina will act as chairman.)

22. Commendation for President Hodgson. Voted that the Executive Board commend the retiring President, R. E. Hodgson, for inspiring leadership during the past year and wish him many years of happy association in the work of A.D.S.A. in the future.

23. Appreciation to retiring Board members. Voted that the Board express its appreciation for the service rendered by retiring members N. P. Ralston and E. C. Scheidenhelm, and wish for them many more years of life and service in A.D.S.A.

24. Solids-not-fat Committee. The Board voted to defer action on a request from the Production and Extension Sections for the appointment of a committee dealing with solids-not-fat, until the mid-year meeting of the Board.

25. Message for Mrs. Jackson. The Board instructed F. J. Doan to prepare a message for

[1] The cost for publishing the Interpretive Summaries in Volume 43 for 1960 was about $3,300. Editor-in-Chief.

Mrs. Howard Jackson, offering the sympathy of the Board in the death of her husband, statement to be transmitted to Mrs. Jackson by the Secretary.

26. Standard Methods for Milk Analysis. The Board reaffirmed its interest in cooperating on this matter with the American Public Health Association and instructed the Secretary to write to the APHA, expressing our interest in working with them.

ACTION ON COMMITTEE REPORTS

1. Journal Management. Voted to accept the report, eliminating the obsolete budget and having the report edited for publication in the JOURNAL.

It is suggested that a symposium at the 1962 meeting be held for dairy economists and marketing specialists.

Voted that the A.D.S.A. Review contain a review of all papers that are published in the JOURNAL OF DAIRY SCIENCE.

Voted that the Journal Management Committee develop the idea of shifting appropriate material from the JOURNAL to the A.D.S.A. Review and within the resources available.

Voted that the Journal Management Committee study our publication policy with the possible publication of a semi-popular journal in addition to the JOURNAL OF DAIRY SCIENCE.

Voted that a survey of reader appeal of the A.D.S.A. Review be held in abeyance for an indefinite period, and review this decision at the next Board meeting in December.

2. Policy Committee. Voted to accept the report of the Policy Committee subject to the actions taken at the Board Meeting.

Voted to refer to the Education Committee the creation of some kind of career pamphlet.

Voted that the item on professional development also be referred to the Education Committee.

Voted that the Award of Honor continue to be restricted to one individual.

Voted that the rules for the Distinguished Service Award be amended to provide for only one award a year instead of one to three.

Voted that Item 4—Program for Annual Meeting—be referred to the Program Committee (both A and B Sections) with the suggestion that serious consideration be given to continue a four-day annual meeting like there will be in 1962.

Voted that the Board adopt a policy about establishing a sustaining membership program and efforts be made to get this done as soon as possible.

Voted to refer to the Policy Committee the policy of life membership, with respect to the impact on the Association finances.

Voted to refer to the Policy Committee the task of reviewing the objectives of the Association for future needs and development and especially the membership qualifications.

3. Rules for Awards. Voted that this Committee be instructed to complete the report as quickly as possible.

4. Nominating Committee. Voted to accept the report.

5. Publicity Committee. Voted to accept the report with commendations.

6. Membership Committee. Voted to accept the report and commend the Committee for their excellent analysis of membership problems in the Association. The Executive Board concurs with the recommendations of the Committee that (1) the same general state organization for membership be retained, (2) that intercommunication between the Secretary-Treasurer and the State Chairman, especially for new members and delinquents, be extended, (3) that the Secretary's method of working through the department heads on Student Affiliates be continued, (4) that the Secretary-Treasurer be commended for contacting members who dropped their memberships.

7. Historian's Report. Voted to accept the report and command him for continued interest, enthusiasm, and diligence in documenting the work of the Association.

It was suggested that the Historian develop a program to publish in the JOURNAL OF DAIRY SCIENCE special biographical sketches of distinguished dairy leaders.

8. Education Committee. Voted to accept the report of the Education Committee and that action be taken to assure appointments to this Committee not later than May 1 of each year, as recommended. The Committee is commended for the development this year of a general sessions program of interest to all segments of the industry. The Executive Board recognizes the importance of the work of this Committee, and that progress may be slow in such a broad area. Therefore, it recommends that the Committee focus attention and concentrate actively on carefully delineated problems of current interest.

9. Public Health. Voted to accept the report of the Public Health Committee with commendations for focusing attention on the problems of mastitis, pesticides, radioactivity in milk, and on the possible relationship between dairy products and heart disease. The Committee's continued alertness to safeguard the healthfulness of the products of the industry is commended.

10. National Research Council. Voted to accept the report with commendation for the representative on the Board of the National Research Council for his excellent report and the effective manner in which he has represented the interests of the Association.

11. Three-A Standards Committees. Voted to accept the report of the A.D.S.A. representatives to the Three-A Sanitary Standards Committees and commends the Committees for making an outstanding contribution to the

dairy industry. The Board concurs in the recommendations of the representatives that their membership insofar as possible be drawn from those already serving an active part in one of the Three-A Committees.

12. Revision of Constitution and By-Laws. Voted to accept the report.

13. National Mastitis Council. Voted to accept the report of the A.D.S.A. representative to the National Mastitis Council and the Association to retain an affiliate with the Council to report on its progress.

14. Program Committee. Voted that the Editor-in-Chief be made a member of the Program Planning Committee. Voted to accept the Program Committee report.

The Board believes that the host institution representative should meet with the Program Committee at the Chicago planning conference to advise about meeting rooms, schedules, and other matters. The Board suggests that the A.D.S.A. President be directed to make appointments of incoming members of the advisor's group of the National Student Branch each year, so that one member is located at the institution where the President of the National Student Branch resides.

The Board did not approve the suggestion that a list of detailed requirements concerning abstracts be published with the Call for Papers, because: (1) This would probably fail to eliminate the problem, (2) cost of publication would not be justified, (3) responsibility lies with author(s), not the Program Committee, (4) a guide for authors will be available.

It is suggested that the General Chairman of the Program Committee and the Section Chairman should use the report of the Program Committee from the previous year as a basis for planning each year.

15. Student Affiliate. Voted to accept the report and approved a method of financing the National Student Branch by charging a fee of $5 per Student Affiliate Chapter. The Constitution of the National Student Branch was accepted as written.

16. Report of Representative to A.A.A.S. Voted to accept the report.

17. Purina Fellowship. Voted to accept the report of the representative of Ralston Purina Research Fellowship Committee and suggest that more dairy departments encourage more students to apply for these awards.

18. N.A.A.B. Report. Voted to accept the report and commend the N.A.A.B. for aiding fundamental research of importance to dairy cattle breeding by providing financial aid.

19. U. S. Livestock Sanitary Association. Voted acceptance of Professor I. D. Porterfield's letter, with the assurance that the Board will receive this report and the representative will recommend what should be published in the JOURNAL.

20. International Relations. Voted to accept the report, that the Committee be continued, and that some specific projects be put before the A.D.S.A. for action. The Board commends the Committee for its fine work.

Voted that A.D.S.A. go on record as favoring the formation of a National Committee to affiliate with the International Dairy Federation, and the Executive Board instructed the International Relations Committee, in cooperation with officers of various associations, to try to formulate a National Committee for this purpose.

Voted that the A.D.S.A. hold in abeyance its decision for holding an International Forum until a decision is reached about affiliating with I.D.F.

Voted that the Committee on International Affairs write a paper dealing with the A.D.S.A. and its foreign relations for members and subscribers and publish it in the JOURNAL.

Voted that the President be empowered to appoint an advisory committee to Dairy Society International (D.S.I.).

21. American Grassland Council. Voted to accept the report of the representative of the A.D.S.A. to this Committee.

22. International Association of Milk and Food Sanitarian Milk and Milk Products Labeling. Voted to accept the report and commend the Committee for its objectives and continue to have a representative.

23. Business Management Committee. Voted to accept Plan Six as the present one and that Plan Two be accepted as a shelf plan. Plan Six is the present procedure, with the possibility of further transferring some of the services performed by the Secretary-Treasurer to the printer of the JOURNAL. Plan Two will employ a full-time Secretary-Treasurer to perform the duties now performed by the Secretary-Treasurer, as well as those that are being performed by the printer of the JOURNAL. The plan also calls for the continuation of a part-time Editor, with all the assistance required to edit the Association publication(s).

REPORT OF THE EXTENSION SECTION

The business meeting of the Extension Section was called to order at 10:30 A.M. on Tuesday, June 13, 1961, Chairman, Marvin Senger.

The officers elected for 1961-62 were W. R. Van Sant, Chairman, Arizona, C. D. McGrew, Vice-Chairman, Ohio, Donald Voelker, Secretary, Iowa.

The four committees of the Extension Section were responsible for developing and presenting portions of the Extension Section program pertinent to their respective interests.

The Teaching Methods Committee, under the chairmanship of James W. Crowley, sponsored a paper by Dr. H. L. Ahlgren, Associate Director of Wisconsin, on An Extension Program in a Changing Agriculture. A panel discussion on A Team Approach to Problem Solving was

moderated by R. E. Burleson of the Federal Extension Service, USDA. Panel participants were M. F. Ellmore, Virginia, J. R. Parrish, Alabama, F. V. Burkalow, Wisconsin, and C. D. McGrew, Ohio. Eight states presented and discussed Extension Exhibits.

Chairman J. E. Cooper of the 4-H Club Program Committee arranged for two program presentations: 1. 4-H Calf Club Projects Can Help Provide Herd Replacements, F. N. Knott, North Carolina; 2. 4-H Club Work for the Sixties, M. S. Downey, 4-H Club Division of USDA.

The Dairy Records Committee, under the chairmanship of W. R. Van Sant, arranged for a panel discussion of Problems of Dairy Cattle Identification. This was moderated by E. C. Meyer of *Hoard's Dairyman*. Other participants were K. Finch, DHIA Supervisor of Wisconsin, Charles E. Bohl of American Jersey Cattle Club, C. L. Pelissier of California, and J. D. Burke of New York. Dr. R. W. Touchberry of Illinois presented a paper on Usefulness and Limitations of Proved Sire and Stable-Mate Records. Dr. J. F. Kendrick of Dairy Cattle Research Branch, USDA, presented a progress report, Present Status of the New Proved Sire Report. In addition, the Dairy Records Committee arranged for four presentations on An Action Program to Improve DHIA Records. The participants were R. W. Fincham, Iowa, C. H. Parsons, Massachusetts, H. C. Gilmore, Pennsylvania, and G. V. O'Bleness, New York.

COMMITTEE REPORTS

Teaching Methods: Extension Section accepted the recommendations of this committee to the effect: (a) that a representative of the host institution serve on the committee for a one-year term, preferably not as chairman; (b) that an effort be made to obtain more bulletins and publications to supplement State exhibits; (c) that abstracts should not be required for invitational papers; (d) that in case of commercial exhibits offered, appropriate Extension Section committees be consulted for their suitability.

4-H Club Committee: The following recommendations were made and accepted: (a) that the method of selecting State 4-H Dairy Judging Teams be left to the discretion of the individual states; (b) that continued support should be given the National 4-H Judging Contests.

Daily Records Committee: Several recommendations were accepted. Among them were: (a) that evaluation of new types of testing procedures and equipment, weighing and sampling equipment, and devices developed for use in production testing be evaluated by special committees from the Production and Manufacturing Sections.

Resolutions: Two resolutions were approved by Extension Section and later received approval in the General Business Section: 1. Commending the Commodity Stabilization Service for assistance rendered in sire proving; 2. Urging increased support to the Dairy Cattle Research Branch to advance dairy herd improvement production testing and sire proving.

The Extension Section took action favoring the establishment of a joint Production-Extension-Manufacturing Section Committee to consider the implications of protein and other solids-not-fat.

A total of twenty-one papers were presented before the Extension Section or joint Production-Section meetings. Abstracts of papers have been published, and copies have been distributed to all Extension Dairymen.

M. E. Senger, Chairman
W. R. Van Sant
C. D. McGrew

REPORT OF THE MANUFACTURING SECTION

Officers elected for 1961 were M. L. Speck of North Carolina State College, Chairman; D. M. Graham of Pet Milk Company, Vice-Chairman; E. L. Thomas of University of Minnesota, Secretary.

The committee to develop a score card for concentrated milk for beverage purposes reported progress. Plans were made for a collaborative study among several laboratories to compare the proposed scoring system with Hedonic ratings. An effort will be made to simplify and standardize the list of flavor criticisms. The committee was continued for another year.

The committee on milk proteins reported further effort in compiling literature related to casein components not previously identified. Purity, homogeneity, and identity of several casein fractions are still uncertain. The committee was largely responsible for developing the protein symposium at the annual meeting. The committee was continued for another year.

The Butter Committee terminated its work concerning formulation of a Standard of Identity for butter. The majority report favored amending the Butter Act of 1923 to keep the 80-per-centum clause only on the statutes and further to seek to obtain a Standard of Identity for butter under the Food and Drug Administration. It was felt this would benefit the butter industry by providing a more flexible standard, whereby the product could be improved with advancing technology. The minority report indicated reluctance to modify the present congressional standard until a specific issue evolved showing undue restriction of the industry by the present standard.

The committee on Judging Dairy Products reported a successful contest with twenty-five teams participating. Three fellowships were awarded by Dairy Industry Supply Association. Rules were modified to provide for grading of a previously drawn cheese plug placed in a

test-tube to help standardize the appearance judging of texture. Significant progress was made towards revision of the several official score cards.

The Resolutions Committee commended the industry for its work in reducing antibiotic contamination in milk and urged continued and vigorous action on this problem.

The Extension Section requested aid in evaluating new equipment and methods related to production testing. Committees will be appointed as need is indicated by the Dairy Records Committee.

BURDET HEINEMANN, Chairman
M. L. SPECK
D. M. GRAHAM

REPORT OF THE PRODUCTION SECTION

The Production Section Business Meeting was held at 11 A.M. on June 13, with Chairman S. D. Musgrave presiding. Minutes of the 1960 meeting were read and approved. Officers elected for 1961-62 were: Chairman, J. C. Thompson (Missouri); Vice-Chairman, L. H. Schultz (Wisconsin); and Secretary, V. R. Smith (Arizona).

The Dairy Cattle Judging Committee report by C. F. Foreman was adopted as presented. It included a recommendation that the Delaware Valley College of Science and Agriculture at Doylestown, Pennsylvania, be granted eligibility to participate in the National Intercollegiate Dairy Cattle Judging Contest, starting in 1961. No action was taken on the question of Eligibility of Contestants, but further study was recommended. Results of a questionnaire sent to the 27 colleges and universities sending teams to the National Contest in 1960 indicated that more than 50% of the 20 replying were in favor of a change, but there were also strong and logical arguments presented against making alterations in the eligibility regulations. The committee reported that a summary of all the actions of the Judging Committee during the past ten years was being compiled and would be completed and distributed prior to the 1961 competition.

The report of the Resolutions Committee by C. L. Norton was adopted. It expressed appreciation to the Section officers, the various committee members, the members of the Dairy Husbandry and Dairy and Food Industries Departments of the University of Wisconsin, and all contributing agencies, for their respective efforts in making the 56th annual meeting informative and enjoyable. An additional resolution presented by J. E. Legates was also adopted. It involved expressing appreciation to the Commodity Stabilization Service of the United States Department of Agriculture for the fine cooperation the New Orleans Commodity Stabilization Services Office has given the Dairy Cattle Research Branch in providing facilities for the automation of the sire-proving procedures.

Chairman Musgrave commented on the rules regarding the preparation of abstracts of papers presented at the annual meeting, indicating that a fairly large number were returned because they did not meet the regulations. He also explained the difficulties involved in placing papers in the proper section, because of the need to put all graduate student competition papers in the program by Tuesday noon.

A motion recommending that the Board of Directors consider appointing a committee on milk solids (emphasizing solids-not-fat), to be made up of two members from each section of the Association, was passed. The purpose of this committee would be to represent the Association in the coordination of research and other activities regarding solids-not-fat. This action was requested by the present solids-not-fat committee with regional representatives. The new committee would not replace or take over any of the activities of the present regional committee, but rather give the Association an official committee in this area which would represent all sections.

A joint meeting of the Production and Extension Sections was held at 4:30 P.M. on June 13. The report on this meeting appears in the Extension Section report.

S. D. MUSGRAVE, Chairman
J. C. THOMPSON
L. H. SCHULTZ

REGISTRATION AT 56TH ANNUAL MEETING OF AMERICAN DAIRY SCIENCE ASSOCIATION

Men:

Members	788
Affiliates	275
Non-Members	199
Women	394
Children	260
Total	1,916

Number of men registering from Universities	901
Other Male Registrants	361

The following foreign countries were represented:

Australia	India
Belgium	Japan
Canada	Mexico
Costa Rica	New Zealand
England	Spain
Holland	Thailand

All states but one were represented.

NEW PRICE SCHEDULES FOR MEMBERSHIPS AND SUBSCRIPTIONS

H. F. JUDKINS
Secretary-Treasurer, White Plains, New York

The Executive Board at the 56th Annual Meeting, at the University of Wisconsin, found it necessary to increase prices for memberships, subscriptions, and single issues of the JOURNAL OF DAIRY SCIENCE. These increases were necessary because of the new A.D.S.A. Review, increased printing costs, increased size of the JOURNAL, and decreased advertising income.

Effective July 1, 1961, single issues of the JOURNAL were increased from $1.25 to $2, except for complete volumes, which will be the same as regular subscriptions.

Effective January 1, 1962, memberships will be increased from $10 to $12.50 per year, domestic subscriptions from $15 to $20, and foreign subscriptions from $15.50 to $20.50 per year.

BOOK REVIEWS

BELOVED PROFESSOR: THE LIFE AND TIMES OF WILLIAM DODGE FROST. Russel E. Frost, Vantage Press, 120 West 31st Street, New York 1, New York.

Pioneer University of Wisconsin bacteriologist; crusader in the fight against tuberculosis; public-health leader; and well-met friend, William Dodge Frost was an inspiration to generations of students.

Beloved Professor is a richly detailed biography of this distinguished scientist and inventor who furthered the progress of American medicine. In writing the book, Russell E. Frost had the co-operation of his noted father in filling in background details of Dr. Frost's eventful life.

W. D. Frost

It was in 1895 that William Dodge Frost, a graduate of the University of Minnesota, accepted an assistantship in bacteriology at the University of Wisconsin and settled into the fullness of his life work. A man whose associates included Robert Koch, who isolated the tubercle bacillus; Selman A. Waksman, discoverer of streptomycin; Dr. Edward Trudeau, founder of the Saranac Sanatorium; Sir William Osler and Dr. William H. Welch of Johns Hopkins, Dr. Frost fought tuberculosis throughout his long and dedicated career.

He invented such boons to bacteriological study as the Frost Little-Plate Method and the Frost Gasometer; Dr. Waksman, considered the father of antibiotics, said, "He laid the basis for our knowledge of the production of specific substances which inhibit the growth of, or destroy, other bacteria." As a second career, he initiated, helped build, and later directed Morningside Sanatorium and was a founder of the University of Wisconsin Medical School.

Written with the wit and humor of a skillful writer, *Beloved Professor* emerges as a portrait of a warm and dedicated man who rejoiced in the achievements of his students. The book is illustrated with photographs and will be of special interest to high-school as well as college students, and teachers in all fields, particularly science.

NUTRITION ANIMALE. Vol. II. Données Généneralés sur la Nutrition et l'Alimentation. Part II. Métabolismes et Transits. R. Jacquot, H. Le Bars, A. M. Leroy, and H. Simonnet.

This book is Part II of Volume II of a series of ten volumes to be published under the title Nutrition Animale as part of the Nouvelle Encyclopédie Agricole, which is being published by J. B. Baillière et Fils, Paris, under the direction of Jean Lefèvre and Pierre Tissot of the Institut National Agronomique. Volume I and Volume II, Part I, have been previously reviewed (J. Dairy Sci., 41, A19. 1958; 43, 1030. 1960).

In this second part of Volume II, the authors discuss the transport of water and of minerals in Chapter V (Chapter I through IV appeared in Part I), the metabolism of vitamins and antibiotics in Chapter VI, and the metabolism of energy in Chapter VII. This is obviously a very wide area to cover in 465 pages. However, in general the review is quite comprehensive and perhaps at times suffers as much from noncritical inclusion of material as it does from omissions.

Chapter V discusses calcium, phosphorus, and magnesium with regard to distribution and chemical forms present in the body and also from the point of view of metabolism. The function of these minerals is discussed, and it is here that one first realizes the limitations in coverage. For example, the role of phosphorus in energy transformations is covered in two pages, while under regulation of transport almost three pages are devoted to the role of vitamin D in calcium and phosphorus metabolism. Certainly, at the moment much more is known about the role of phosphorus in energy metabolism than about the role of vitamin D in calcium and phosphorus metabolism.

Under the general headings of (a) tissue distribution, (b) metabolism, (c) function, and (d) regulation of transport are discussed iron, copper, cobalt, sulfur, iodine, manganese, molybdenum, fluorine, and zinc, together with a long list of trace elements. A short section is included on the role of iron, copper, and cobalt in hematopoiesis, which correctly attributes the role of cobalt in the prevention of anemia in the ruminant to the requirement of the rumen flora for cobalt in the biosynthesis of vitamin B_{12}, the hematopoietic factor involved.

Chapter VI, dealing with the metabolism of the vitamins and antibiotics, discusses vitamins A, D, E, K, thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, cobalamin, and ascorbic acid under the headings: (a) forms and states in the organism, (b) metabolic role, and (c) functional role. Under functional role the authors include deficiency symptoms and hypervitaminosis.

The 30 pages devoted to antibiotics discuss practical uses of antibiotics in animal nutrition and their role in the sparing of protein, vitamins, and minerals, and theories of mechanism of action. In discussing the relationship between antibiotics and B vitamins, the

authors continue to speak of a sparing action of the antibiotics and have neglected the recent work showing the site of action of the antibiotics with regard to B-vitamin synthesis.

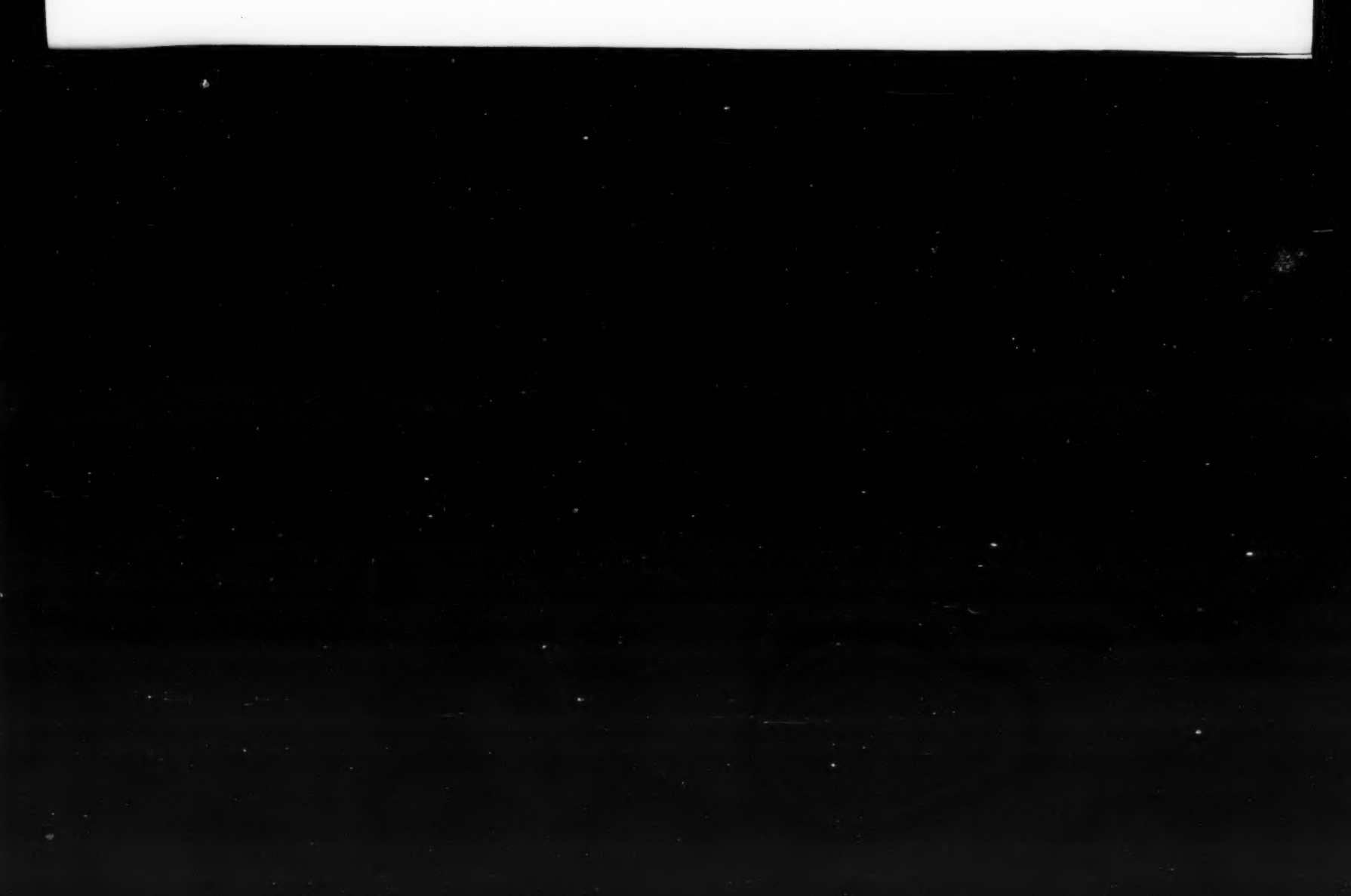
Chapter VIII on energy metabolism occupies about one-third of the volume of the book and illustrates the recent revival and interest in energy work in Europe.

B. C. JOHNSON
University of Illinois, Urbana

THE IN-PLACE CLEANING MANUAL. Published by the Society of Dairy Technology, 17, Devonshire Street, London, W.1. Price 10s, including postage.

This manual will be useful to plant managers, plant superintendents, and to regulatory personnel in the various public health services. It includes an account of the basic principles involved in in-place cleaning and the essentials in maintaining its efficiency. This book is practical and includes diagrams of plant lay-outs, methods of operations, and a sound theoretical background of information. This manual is an objective appraisal of in-place cleaning—its advantages and pitfalls.

E. O. HERREID
University of Illinois, Urbana



A MATTER OF SOME CONCERN

What Is the Official Plating Medium?

THE ANSWER to this question is a matter of immediate concern to State Laboratory Directors, Milk Laboratory Survey Officials, and other dairy laboratories. There have been many conflicting reports and misunderstandings as to the current status of Standard Plating Medium.

Standard Methods for the Examination of Dairy Products, 11th edition 1960 (APHA) describes the Agar Plate Method and the materials to be used. This compendium specifies the use of Standard Methods Agar (Plate Count Agar) conforming to the formula appearing in the appendix (34) or one "giving equivalent" results. It goes on to say, "Before official use as a basis for determining Standard Plate Counts, all ingredients alleged to give 'equivalent results' (3.07a) should have unqualified approval for such use by the Sub-committee on Standard Methods for the Examination of Dairy Products of the APHA."

No official compendium, Standard Methods, 11th edition, USP or AOAC stipulates that, "official use" recommends a medium that is certified by the Microbiological Media Commission. Neither does the United States Public Health Service or any other official agency recommend the use of certified media. This presumed condition and recommendation or requirement has been grossly misrepresented.

The Microbiological Media Commission is a privately owned and operated corporation. It has no official connection with the American Public Health Association, although it is mentioned in Standard Methods, 11th edition. This reference is clearly qualified by a footnote stating that certification procedure had not become functional and certified media would not be available until a program is implemented that is acceptable to the APHA.

The American Public Health Association has not implemented a program that recognizes the Microbiological Media Commission or certified media. The only official plating medium is STANDARD METHODS AGAR as described in Standard Methods for the Examination of Dairy Products, 11th edition 1960 and as Tryptone Glucose Yeast Agar in the USP and AOAC. In addition to specifying the ingredients, Standard Methods further specifies performance to meet productivity standards as described in the American Journal of Public Health, 44:935, 1954.

We hope this information will serve to clarify the status of official plating media and will assist the milk laboratories in selecting a brand of Standard Methods Agar (Bacto-Plate Count Agar) that meets all prescribed standards of quality and performance.

DIFCO LABORATORIES

DETROIT 1 MICHIGAN USA